3T magnetic resonance imaging of cortical grey matter lesions in Multiple Sclerosis.

Dissertation submitted for degree of
Doctor of Philosophy

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Declaration

I, Dr Varun Sethi confirm that the work presented in this thesis is my own. Where information has been contributed from other sources and by colleagues, I indicate this in the Acknowledgements section.

Abstract

Radiological and histopathological studies have established that in addition to the classical white matter (WM) demyelinating lesions, cortical grey matter (CGM) lesions are also a significant part of the pathology in multiple sclerosis (MS), and contribute to the clinical and cognitive deficits seen in MS patients. Double inversion recovery (DIR) has been identified as a good sequence for the radiological detection of cortical grey matter lesions.

In this project I investigated the role of phase sensitive inversion recovery (PSIR), a T1-weighted MRI sequence, using a 3T MRI scanner, for the detection of CGM lesions in multiple sclerosis. Detection of CGM lesions on a standard DIR sequence (1x1x3mm resolution) was compared with a higher resolution PSIR sequence (0.5x0.5x2mm), to explore if it can help improve the study of CGM lesions. A representative cohort, including patients with relapsing remitting (RR), primary progressive (PP) and secondary progressive (SP)MS, was recruited in this project, together with controls in order to allow a comparison of findings with those of healthy subjects who do not have MS.

I systematically investigated if the use of high resolution PSIR scans can improve CGM lesion detection and classification, when compared to DIR. Using the PSIR sequence, I studied the hypothesis that the distribution of lesions impacts the pattern of cognitive impairment seen in patients. CGM lesion volumes were estimated for frontal, temporal , parietal and occipital lobes and cognitive tests were conducted (Hayling, Stroop, immediate and

delayed story and figure recall, PASAT (Paced auditory serial addition test) and SDMT (Symbol digit modality test)). Differences between phenotypes and associations with cognitive measures were explored using a multiple regression model. A follow up study was undertaken to understand how CGM lesions evolve with time and in order to explore potential specificity of PSIR-detected CGM lesions, I compared the findings of CGM lesion detection in MS patients, with patients diagnosed with Fabry's disease.

Compared with DIR, high resolution PSIR was found to detect a significantly greater number of CGM lesions and also improved the classification of CGM lesions. A fronto-temporal dominance of CGM lesions was noted in my study. Different CGM and JC lesion subtypes were found to be associated with cognitive function; the relationship being influenced by the lobar location and the cognitive function being assessed. In the follow-up study I found that people with SPMS have a grater accrual of CGM lesions than RRMS and the process appeared to be independent of WM lesion accrual. CGM lesions were also seen in patients with Fabry's disease though the frequency was less that in MS.

The data presented in my study suggests that PSIR has the potential to improve the quantitative and qualitative study of CGM lesions. CGM lesions were noted across all disease phenotypes, though more common in progressive disease. The distribution, accrual and evolution of CGM lesions provides insights into the pathogenesis of MS and helps understand the contribution of CGM lesions to neurological and cognitive impairment. Detection of CGM lesions has a potential role to help with a diagnosis of MS when it suspected but not confirmed.

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Publications arising from this work

As first author

- Sethi V, Yousry TA, Muhlert N, Ron M, Golay X, Wheeler-Kingshott CAM, Miller DH Chard DT. (2012). Improved detection of cortical MS lesions with phase sensitive inversion recovery MRI. Journal of Neurology, Neurosurgery & Psychiatry, 83 (9): 877-82.

- Sethi V, Muhlert N, Ron M, Golay X, Wheeler-Kingshott CA, Miller DH, Chard DT, Yousry TA. (2013). MS Cortical Lesions on DIR: Not Quite What They Seem? *PLoS One*: e78879.

As co-author

- Muhlert N, Sethi V, Schneider T, Daga P, Cipolotti L, Haroon HA, Parker GJM, Ourselin S, Wheeler-Kingshott CAM, Miller DH, Ron MA, Chard DT. (2013) Diffusion MRI-based cortical complexity alterations associated with executive function in multiple sclerosis. *Journal of Magnetic Resonance Imaging*, 38 (1): 54-63.
- Samson RS, Muhlert N, Sethi V, Wheeler-Kingshott CAM, Ron M, Miller DH, Chard DT. (2013). Sulcal and gyral crown cortical grey matter involvement in multiple sclerosis: a magnetisation transfer ratio study. *Multiple Sclerosis & Related Disorders*, 2 (3): 204-212.
- Samson RS, Cardoso MJ, Muhlert N, Sethi V, Wheeler-Kingshott CA, Ron M, et al. (2014). Investigation of outer cortical magnetisation transfer ratio abnormalities in multiple sclerosis clinical subgroups. *Multiple Sclerosis, 20*(10), *1322*–1330.

Abbreviations

25 TWT	25 foot timed walk test
2D	2 Dimensional
3D	3 Dimensional
3T	3 Tesla
7T	7 Tesla
9.4T	9.4 Tesla
9HPT	9 hole peg test
ACE	Angiotensin converting enzyme
BBB	Blood brain barrier
BRNB	Rao Brief Repeatable Neuropsychological Battery
C	Controls
C'	Complement
CD	Cluster of differentiation
CGM	Cortical grey matter
CI	Cognitive impairment
CIS	Clinically isolated syndrome
CNR	Contrast to noise ratio
CNS	Central nervous system
CSF	Cerebrospinal fluid
DIR	Double Inversion Recovery
DIS	Dissemination in space
DIT	Dissemination in time
DMT	Disease modifying therapy
DTI	Diffusion tensor imaging
EBV	Epstein-Barr virus
EDSS	Expanded disability status scale
F	Female
FLAIR	Fluid attenuated inversion recovery
FOV	Field of view
GAD	Gadolinium containing MRI contrast agents
GE	Gradient echo
GLA	Galactosidase alpha
GM	Grey matter
GMF	Grey matter fraction
IC	Intracortical
ICV	Intracranial volume
IR	Inversion recovery
JC	Juxtacortical
LC	Leucocortical
LPM	Lesion probability mapping
M	Male
MACFIMS	Minimal Assessment of Cognitive function in MS

MBP	Myelin basic protein
MDP	Myelin degradation products
mins	minutes
mm	millimeter
MPRAGE	Magnetisation prepared rapid gradient echo
MRI	Magnetic resonance Imaging
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
ms	milliseconds
MS	Multiple sclerosis
MSFC	Multiple sclerosis functional composite
MTR	Magnetisation transfer ratio
NAGM	Normal appearing grey matter
NAWM	Normal appearing white matter
NK	Natural killer cells
NMO	Neuromyelitis optica
Non JC WM	Non juxtacortical WM
OCB	Oligoclonal bands
PASAT	Paced auditory serial addition test
PD	Proton density
PPMS	Primary progressive multiple sclerosis
PSIR	Phase sensitive inversion recovery
RF	Radiofrequency
RIS	Radiologically isolated syndrome
RRMS	Relapsing remitting multiple sclerosis
SD	Standard deviation
SDMT	Symbol digit modality test
SE	Spin echo
SEL	Slowly expanding lesion
SENS	Sensitivity encoding factor
SLE	Systemic lupus erythematosus
SNR	Signal to noise ratio
SPIR	Spectral presaturation inversion recovery
SPMS	Secondary progressive multiple sclerosis
SWI	Susceptibility weighted imaging
T	Tesla
T1w	T1 weighted
T2w	T2 weighted
TE	Echo time
TR	Repetition time
VR	Virchow Robin spaces
WM	White matter

1 Introduction

1.1 Background

Multiple sclerosis (MS) is a chronic neuro-inflammatory condition that causes demyelination and neuronal loss in the central nervous system (CNS). The pathological hallmark, focal CNS white matter (WM) lesions, were first depicted in 1838 by Carswell (Leary et al. 2009) and the condition was studied further by Charcot who called it sclerose en plaques (Frohman 2006; Polman et al. 2005; Rinaldi et al. 2011; Compston 1999). The understanding of the clinical features, natural history and pathogenesis of MS has improved over the years, and this is reflected in the improved sensitivity and specificity of the diagnostic criteria for MS, most recently revised in 2010 (Polman et al. 2011).

Based on the natural history of disease and the duration and progression of clinical symptoms, four phenotypes of MS are recognised. These are relapsing remitting, primary progressive, and secondary progressive and progressive relapsing MS (Leary et al. 2009; Lublin & Reingold 1996). In relapsing remitting MS, the clinical course consists of relapses (episodes of neurological dysfunction with partial or complete recovery) that are caused by focal inflammatory events; neurodegenerative mechanisms are likely the main cause of progressive disability that accrues in later stages of relapse onset disease (secondary progressive MS)(Trapp et al. 1999).

The pathological features of MS, consist of white matter lesions that represent areas of demyelination with relative sparing of axons and varying

degrees of inflammation. The common sites of dymyelination include optic nerves, corpus callosum, periventricular regions, brainstem, cerebellum and the spinal cord. The normal appearing white matter (NAWM) is also seen to show abnormalities (Mistry et al. 2011; Kutzelnigg et al. 2005a; Zeis et al 2008; Graumann et al. 2003).

Though traditionally thought to be limited to the white matter (WM), Charcot (as early as 1868) had pointed out that in addition to cerebral and cerebellar WM, deep grey matter (DGM), and spinal cord white and grey matter, cortical demyelination was also present in MS (Popescu et al. 2012a). Histopathological observations in 1898 (Sander) and 1916 (Dawson 1916) also reported involvement of grey matter (GM) in regions in MS. In routine bipotic and autoptic pathology, the conventional lipid stains that were used to study demyelinated lesions were unable to prove useful in regions of low myelin density, as in the cortex and deep grey matter (Stadelmann et al. 2008).

In addition, cortical pathology was also underestimated due to the scarcity of obvious tissue damage, gliosis and minimal perivascular inflammatory infiltrate. Immunohistochemistry of MBP (Myelin basic protein) (Brownell & Hughes 1962; Lumsden 1970; Bø et al. 2003b; Bø et al. 2009; Geurts et al. 2008) and more superior microscopy methods have facilitated the identification of cortical lesions and allows characteristics such as their size and extent to be further explored across all stages and phenotypes of disease. (Stadelmann et al. 2008; Peterson et al. 2001, Bø, 2006a; Vercellino 2005,Kampman et al. 2012, Bø & Bø 2009; Runia et al. 2012; Disanto et al. 2012) It is now established that extensive cortical demyelination is present in

people with MS, and is at least partially (Geurts & Barkhof 2008) independent of white matter pathology (Kutzelnigg et al. 2005a).

In addition to demyelination of axons, grey matter damage consists of neuroaxonal degeneration(Geurts & Barkhof 2008). Focal WM lesions are unable to completely explain the patterns of clinical and cognitive impairment in people with MS. Furthermore, cognitively preserved people with MS have less damage to the cerebral cortex(Amato et al. 2004), suggesting that pathological processes in the grey matter may be the missing piece of the puzzle (Calabrese, Rinaldi, Poretto, et al. 2011b) of the clinical-radiological paradox (Barkhof 2002a).

Histopathological studies of grey matter pathology are possibly significantly skewed by lack of the availability of tissue samples from people across different stages of disease; most samples are either from patients with atypical clinical presentations or post mortem (from people with progressive and long duration of disease). There are practical limits on the amount of tissue that can be assessed and examination of the whole brain is prohibitively time consuming. Longitudinal studies are also not practical using histopathological specimens. Radiological observations on the other hand allow an in vivo study of cortical pathology non-invasively. Scans can be repeated over time, across all subtypes and duration of MS.

Conventional magnetic resonance imaging (MRI) in MS has focused on white matter lesions (Ceccarelli et al. 2012a; Poser et al. 1983; Tintoré et al. 2003). T1-weighted, T2-weighted and fluid attenuated inversion recovery (FLAIR) are the main sequences used. Radiological demonstration of white matter lesions plays an important role in the diagnosis of MS, which requires

the demonstration of disease activity to be disseminated in space and time. This is based on clinical history, together with laboratory and imaging evidence of demyelination, in the form of white matter lesions (Lezak et al. 2012; Griffin et al. 2002; Lucchinetti et al. 1996; Pirko et al. 2007; Allen et al. 2001; Polman et al. 2005; McDonald et al. 2001; Poser et al. 1983; Tintoré et al. 2003). WM lesions appear as hypointense lesions, the so called “black holes” in T1w scans and as hyperintensities in T2w and FLAIR images. There is evidence that T1-weighted black hole lesions are biased towards more destructive lesions and when persistent, represents areas of permanent axonal loss (Haller et al. 2009; van Waesberghe et al. 1999); however findings on these conventional scans cannot be regarded as pathologically specific. Black holes that enhance following contrast injections are young lesions, associated with inflammation and breakdown of the blood-brain barrier, and the hypointensity may reverse with follow up. MS lesions are seen as hyperintense regions with a high signal foci on T2 weighted imaging (Trip & Miller 2005). Oedema, demyelination, axonal loss, oligodendrocyte loss and remyelination (Barkhof et al.2003) are thought to contribute to the hyperintensities seen on T2w imaging. Proton density(PD) weighted images are specially helpful in appreciating periventricular lesions as the adjacent CSF has a lower signal on this sequence (Trip & Miller 2005). These scans are limited in the ability to detect abnormalities in the normal appearing white matter.

Detection of cortical grey matter (CGM) lesions has, however, remained limited using conventional MRI sequences (Geurts et al. 2008; Kidd et al. 1999). Visibility of these lesions is limited by their location, size and

intrinsic properties of the lesions (minimal inflammation, lack of a blood brain barrier disruption (van Horssen et al. 2007) and a lesser degree of contrast between the lesional /non-lesional cortex) (Geurts et al. 2011; Geurts, Bø, et al. 2005a). The proximity of the lesions to the CSF also causes susceptibility artefacts. (Schmierer et al. 2010)

Due to the challenges in detecting cortical lesions, various techniques aimed at global GM assessment have been used (Geurts & Barkhof 2008).These include quantitative measures such as magnetisation transfer ratio (MTR) (Griffin et al. 2002), T1 relaxation time measures (Geurts & Barkhof 2008; Barkhof 2002b; Vrenken, Geurts, et al. 2006a), diffusion tensor imaging (Vrenken, Pouwels, et al. 2006b) and quantification of metabolites such as N acetyl-aspartate using spectroscopy (Chard et al. 2002).

To improve the study of cortical lesions different methodologies have been investigated in an attempt to non-invasively reproduce information derived from histopathological studies, and study the clinical relevance of cortical pathology. The use of MRI with high and ultra-high field strengths, imaging using 3D protocols and using sequences with improved contrast and signal to noise ratio (SNR) are some of these approaches. Ultra-high field strengths beyond 3T (Wattjes et al. 2009) e.g.7T (Nielsen et al. 2013; Absinta et al. 2013; Tallantyre et al. 2009) and 9.4 T (Schmierer et al. 2010; Filippi et al. 2013) provide a higher SNR and thus a greater resolution(Geurts et al. 2008a). Improved contrast between lesion and non-lesional tissue are other advantages. Nevertheless these are not without disadvantages such as problems difficulties of narrow bore and incomplete coverage with some sequences and limited clinical availability. Subpial demyelination (Bø,

Vedeler, Nyland, Trapp & Mørk 2003b) is well recognized in MS but is challenging to detect using MRI. Using imaging at ultra-high field strengths of 9.4 Tesla, it has been possible to visualise subpial cortical demyelination. Schmierer et al. found a total of 36 CGM lesions in the 21 tissue blocks studied, and 18/36 were reported to be Type III (supbial) lesioins. (Schmierer et al. 2010).

An alternative strategy to improve SNR is to use 3D imaging. While in 2D imaging, each radiofrequency (RF) pulse is seen to excite a narrow slice, in 3D imaging, the entire imaging volume is excited by the RF pulse. (Johnson et al. 1999) Using 3D sequences an improved SNR can be achieved in shorter time spans. However in some conditions 2D sequences may in fact be better and more sensitive (Johnson et al. 1999) and various factors such as the slab thickness, the coil used and the sequence parameters need to be considered.

MRI sequences that have been used for the study of cortical lesions include 2D DIR, multi-slab 3D DIR (Geurts, Pouwels, et al. 2005b), single slab isotropic 3D DIR (Geurts & Barkhof 2008)MPRAGE (Nelson et al. 2008), 3D FLAIR (Kilsdonk et al. 2013) and Flash T2* (Nielsen et al. 2011). Double inversion recovery, which suppresses signal from both CSF and white matter, has greatly improved the visualisation of grey matter lesions.(Q. Li et al. 2011; Wattjes et al. 2007; Bender & Klose 2010; Geurts, Pouwels, et al. 2005b; Calabrese et al. 2007). Consensus recommendations for detecting CGM lesions have recently been published (Geurts et al. 2011). Using DIR, it has been possible to detect cortical lesions even in patients with clinically isolated syndrome and radiologically isolated syndrome (Filippi et al. 2010b;

Papadopoulou et al. 2013). It has been possible to separate CGM lesions as being intracortical (IC – limited to the substance of the cortex) and leucocortical (LC – mixed GM/WM lesions) (Geurts et al. 2005a; Geurts & Barkhof 2008; Geurts et al. 2011). However subpial lesions have not been identified using DIR at clinically used field strengths (Geurts et al. 2011; Schmierer et al. 2010). It has also not been possible to accurately separate juxtacortical (JC – white matter lesions that abut but do not enter the cortex) lesions from LC lesions. (Giorgio et al. 2011; Kidd et al. 1999; Geurts, Bø, et al. 2005a). DIR has improved the radiological detection of cortical lesions as compared to FLAIR. The improvement of DIR vs. FLAIR for LC lesions was a sensitivity of 83% (vs. 65%) and on retrospective scoring this was 96% (vs. 91%). However, postmortem correlations indicate that only about 18% of the pathologically visible lesions are visualised (Seewann et al. 2012)

Phase sensitive inversion recovery (PSIR) (Hou et al. 2005),(Nelson et al. 2007a) is a T1-weighted sequence, with a high SNR and good grey - white contrast; it has the potential to improve the quantitative and qualitative detection of cortical lesions. DIR and PSIR, individually and together, have the potential of enhancing the understanding of cortical pathology in MS, across all stages of disease (Nelson et al. 2007a).

Given the differences and limitations of DIR and PSIR, it is important to determine if these two sequences identify similar or different pathological processes and determine if they preferentially identify lesions at a particular location or region of the cerebral cortex. Inflammatory cortical demyelination is common in early MS but in long standing progressive patients, the inflammatory component is not seen typically (Lucchinetti et al. 2011). DIR

does not detect all cortical lesions (Geurts et al. 2005a) and it has been suggested that only the relatively new, oedematous lesions might be visible on DIR (Calabrese et al. 2007b). It remains to be investigated if similar findings are seen with PSIR or whether the latter is more sensitive to detecting lesions even in the absence of inflammation.

The differential specificity to pathological processes has been noted with the use of conventional scanning of WM lesions (van Waesberghe et al. 1998; Guttmann et al. 2006). No MRI sequence on its own is able to provide perfect contrast specific for a particular pathological substrate. The overlap in histopathological specificity is largely due to the distribution of water in the diseased parenchyma (Guttmann et al. 2006). T2 weighted MRI is sensitive to inflammation, oedema, demyelination, loss of axons and to remyelination (Guttmann et al. 1995). The hypointensities on T1w MRI represent axonal loss but this cannot be differentiated from the transient oedematous changes in acute lesions (van walderveen et al. 2001). Diffusion MRI is sensitive to WM damage and magnetisation transfer imaging has an enhanced specificity towards demyelination (Guttmann et al 2006). A systematic combination of different sequences can be used to provide a better understanding of MS pathogenesis. DIR is sensitive to cortical demyelination (Seewann et al. 2012); combined radiological and histopathological studies will help ascertain the pathological substrate of PSIR lesions. If DIR and PSIR are in fact differentially sensitive for different pathological processes, it would suggest a multimodal approach (Ceccarelli et al. 2012a; Tallantyre et al. 2010; Mike et al. 2011; Nelson et al. 2011) combining both DIR and PSIR (Nelson et al. 2007a) would help identify different stages of cortical pathology.

Recently published consensus recommendations (Geurts et al. 2011) (See Table 1.1) advise caution while marking regions high signal in the archicortex and paleocortex e.g. insula. Are there similar or different artefact prone regions on PSIR , and if so, can these scans be used together ? Alternatively can either provide as much information as the other? With respect to the location of lesions, juxtacortical lesions have not been reported separate from LC using DIR. Given that JC lesions have independently been used for diagnosis and to predict cognitive impairment, it will be interesting to see if PSIR improves identification of JC lesions and advance the understanding of cognitive problems in MS.

Table 1.1 Recommendations for marking cortical lesions on DIR

Mandatory features	Cortical lesions are hyperintense on DIR images and should occupy at least 3 pixels (at 1mm^2 isotropic in-plane resolution)
Supportive points	To be aware of regions prone to artefacts - including the archicortex and paleocortex e.g. insula
	Maintain near constant image contrast setting
	Multiple slices of the scan should be seen to ascertain if a hyperintensity is a cortical lesions; this helps differentiate it from artefacts such as vessels
	Use other T1 based and T2 / FLAIR images to confirm doubtful lesions

(Based on Geurts et al. 2011)

The detection and study of CGM lesions has a potential role in (i) diagnosing and differentiating MS from other conditions (ii) understanding the pathogenesis and (iii) correlation between CGM lesions and disability in people with MS. White matter lesion load and distribution does not correlate well with disease phenotype, stage of disease or extent of disability seen in patients. Associations between WM lesion load and presentations such as fatigue (Bakshi 2003) and cognitive impairment (Hulst et al. 2013; Giorgio & De Stefano 2010)are modest. Cortical lesion loads have also been demonstrated to correlate with tests of cognitive impairment. (Calabrese et al. 2009; Calabrese et al. 2011a). If the numbers and distribution of CGM lesions has a better correlation with markers of disability, this will help to bridge the clinical radiological paradox. (Leary et al. 2009; Frohman 2006; Chard et al. 2010; Crawford et al. 2004; Bobholz & Rao 2003; Zang et al. 2004; Giorgio & De Stefano 2010; Trapp & Nave 2008; Jongen et al. 2012).

Cortical lesions, if specific for MS, may have a diagnostic role (Filippi et al. 2010b). However, to be able to establish this, it is important to investigate for evidence of grey matter lesions in healthy controls (to understand whether aging is associated with cortical lesions in the way that it is with white matter lesions) and also in other neurological conditions, such as small vessel disease and migraine, which are also known to cause white matter lesions.

Cortical lesions have been reported in healthy controls with DIR (Geurts et al. 2005b) albeit few. Further studies are needed to determine if these observations in controls are true or represent a misinterpretation of artefacts. If intracortical lesions are found to be specific to MS, they could be

incorporated in the diagnostic criteria for MS (Filippi et al. 2010b)and help improve the specificity of these criteria. While consensus recommendations have been recently outlined for the detection of grey matter lesions on DIR (See Table 1.1) (Geurts et al. 2011), there are no available guidelines for lesion detection using PSIR.

The pathogenesis of grey matter lesions may differ from that of white matter lesions. While there are similarities e.g. both grey and white matter lesions are seen in early stages of disease (e.g. in CIS), (Simon et al. 2012); the relative lack of inflammation and greater extent of demyelination in cortical lesions (vs. WM) are some important differences. Longitudinal MRI studies of CGM lesion evolution may help understand the pathogenesis of GM and WM lesions, and further define new treatment endpoints for clinical trials. A better method to in vivo detect grey matter lesions will likely improve the ability to correlate and predict disease progression in patients.

The sections below describe the etiology, clinical features and pathogenesis of Multiples sclerosis. A background of basic MRI physics and the sequences that have evolved for the detection of grey matter pathology are described. In this project I have also studied the association of cortical lesion load with the performance on cognitive tasks; thus the cognitive tests are described. A brief overview of the cognitive impairment seen in MS is detailed in the subsequent sections.

1.2 Etiology

No definite cause for MS has been found, however a variety of environmental factors may predispose a person with genetic susceptibility, to develop MS (Ceccarelli, Bakshi, et al. 2012a; Gandhi & Weiner 2012). MS is

relatively common in the young population of temperate regions such as northern Europe, North America and Australasia, and less common in Asia and the Middle East (Compston et al. 2006). In the UK, it is estimated that the prevalence of MS is 203.4 cases per 100000 population, (Mackenzie et al. 2014) and the incidence is 9.64 cases per 100000/year. It commonly presents at 20-40 years of age and is more common in females than males; males > females 2:1. (Leary et al. 2009).

Genetic susceptibility for MS has been studied and the major risk factor for MS is a haplotype within the major histocompatibility region; more than a hundred additional loci with genome-wide significance have now been identified but the variants and their effects at molecular and cellular levels are still being understood. (IMSGC et al. 2007; Kemppinen et al. 2011). Ethnicity, gender, and familial risk also influence the chances of developing MS (Nelson et al. 2012).First degree relatives have a 10 to 25 times greater risk of developing MS. (Griffin et al. 2002; Filippi et al. 2010b; Trapp & Nave 2008; Nelson et al. 2007b; Gandhi & Weiner 2012). In comparison to African-American population, the white population is more susceptible and so are women in comparison to men (2F:M). Differences in gender distribution are, however, noted depending on specific phenotype and also based on age at onset and seem to be changing with time. (Compston et al. 2006)

Environmental factors including climate, infections, vaccinations, smoking and diet have all been found to be risk factors for the development of disease (Lauer 1995; Steiner 1952, Martinelli et al 2014; Fernandes de Abreu et al 2011; Runia et al 2012). Infections associated with the development of MS include EBV (Sundström et al. 2004), retroviruses, human herpes virus 6.

Environmental factors like growing up in a temperate climate, being born in summer (and thereby spending in-utero period largely in winter), lack of sunlight exposure and low vitamin D levels (Fernandes de Abreu et al. 2011; Kampman et al. 2012; Runia et al. 2012; Disanto et al. 2012) have all been studied as risks in the context of MS.

1.3 Clinical Aspects

Clinical Features

The clinical hallmark of MS presentation is clinical features disseminated in space (site of localization) and time and the condition was thus formerly known as Disseminated Sclerosis.

Four distinct phenotypes have been described based on the onset and progression of disease.(Leary et al. 2009) These are Relapsing-remitting, Primary progressive, Secondary progressive and Progressive relapsing MS.(Leary et al. 2009; Lublin & Reingold 1996) (See Figure 1.1). The course of disease is very individual and variable between patients. While some patients have a very aggressive course, others run a very mild course e.g. benign MS, wherein patients do not enter the progressive form of disease.

The onset of disease is seen to be relapsing remitting in about 85% patients (Leary et al. 2009) (Nelson et al. 2008; Geurts et al. 2011; Trapp & Nave 2008). An episode of acute or subacute new neurological deficit or worsening of old symptoms, which lasts for a minimum of 24 hours, is defined as a relapse. Symptoms evolve over days and recovery can take up to many weeks. The is very variable; annual relapse rates of 0.1 to more than 1 a year have been reported in different studies (Confavreux et al. 2014; Myhr et al. 2001). Pathologically these clinical events represent focal inflammation and

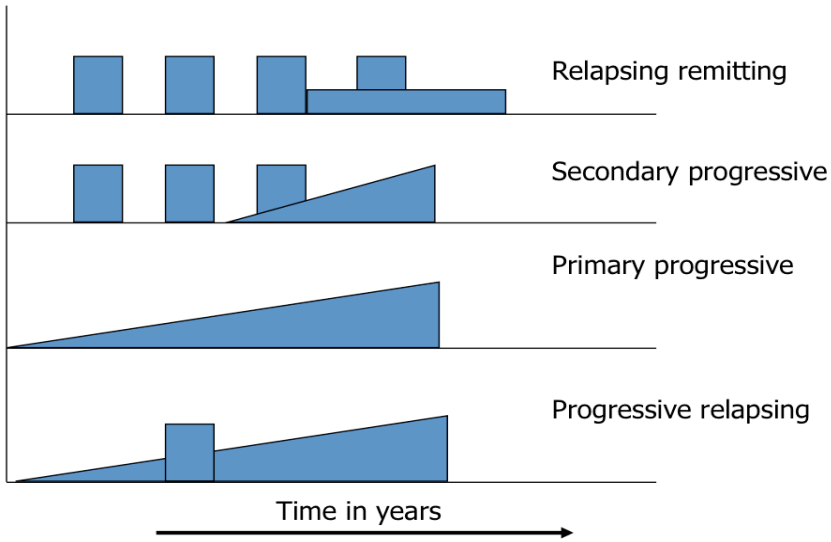
demyelination in the central nervous system white matter. (Seewann et al. 2012; Gandhi & Weiner 2012; Patzold & Pocklington 1982)

In the early years of disease, the incidence of a relapse is estimated to be 1.1 times / year and this decreases with increasing duration of disease and advance to progressive disease.

When recovery following relapses is followed by a gradually increasing, accumulating deficit and disability, the disease is said to enter a secondary progressive phase. About 60% patients progress to SPMS in about 10-15 years after the onset of disease. (Geurts et al. 2011; Weinshenker et al. 1989; Schmierer et al. 2010)

PPMS, on the other hand, has a progressive course from the onset of disease, and about 15% patients present with this pattern. About 25% of patients initially diagnosed to have PPMS, are seen to have superimposed relapses together with the progressive phase of disease and are referred to as progressive relapsing MS.

Figure 1.1 Phenotypes of Multiple Sclerosis



(Adapted from Leary et al. 2009)

Diagnosis of MS

MS (MS) is a neuro-inflammatory condition that has until recently, been thought to be a white-matter disease. The diagnosis of MS is based on a combination of clinical and radiological or laboratory evidence of demyelination; white matter 'lesions' being the radiological hallmark.

Over the years, the diagnostic criteria for MS have evolved from the initial Schumacher criteria (Schumacher et al. 1965) followed by Poser criteria (Poser et al. 1983), McDonald with revisions in (2005)(Polman et al. 2005) and most recently, the 2010 McDonald criteria arose following revisions made by the International Panel for Diagnosis of MS when it met in Dublin in May 2010 (Kilsdonk et al. 2011; Polman et al. 2011); with each iteration showing an increasing trend to gravitate towards the use of laboratory (e.g. CSF analysis) and radiological evidence to aid in the diagnosis

The revised criteria have aimed to maintain the diagnostic specificity and sensitivity while allowing for a simplification – a single scan after a single clinical episode can now at times suffice to establish a diagnosis of MS. (Polman et al. 2011) The use of these criteria allows diagnosis to be established early and thus prompt initiation of treatment, where indicated. Dissemination of disease in space and time remains the essential requirement in establishing a diagnosis. (Popescu & Lucchinetti 2012a; Schumacher et al. 1965; Poser et al. 1983; Schumacher et al. 1965)The use of visual evoked potentials (indicating optic nerve damage), demonstration of unmatched oligoclonal bands, IgG index in CSF (evidence of immunoglobulin generation in the central nervous system), and investigations that help exclude differentials (e.g. Aquaporin 4 antibodies in NMO; Serum and CSF ACE levels

in neurosarcoid; Anti Rho and Anti La antibodies in SLE etc.) may all aid the diagnosis of MS. (Charil et al. 2006)

Radiological criteria are based on demonstration of white matter ‘plaques’ or ‘lesions’ in regions and patterns that are typical of MS: these include the periventricular and juxtacortical white matter, infratentorial (brain stem and cerebellum) and spinal cord.

The use of contrast (gadolinium chelate (GAD)-based) enhanced images helps to (i) exclude other conditions e.g. sarcoidosis which could have accompanying meningeal enhancement and tend to have a persistence of enhancement and (ii) determine the ‘age’ of a lesion; new MS lesions show GAD enhancement, indicating the active, inflammatory state, for about 4 weeks.

It needs to be remembered however, that the diagnostic criteria outlined above should be used only in presentations suggestive of MS / a demyelinating event involving the CNS, the first such being referred to as a clinically isolated syndrome (CIS) i.e. a single isolated demyelinating event. These presentations can remain localized to one neuroanatomical location (monofocal) or involve multiple sites (multifocal), at the same time. Some common presentations of CIS include optic neuritis, partial myelitis or involvement of the brainstem/ cerebellum/ cerebral hemispheres. (Polman et al. 2011; Miller et al. 2012; Miller et al. 2005).

The 2010 McDonald MRI Criteria for dissemination in time and space are outlined in Table 1. (Montalban et al. 2010; Polman et al. 2011). To demonstrate dissemination in space (DIS), one or more T2 lesions are required to be seen in at least two regions amongst the periventricular,

juxtacortical, infratentorial and spinal cord regions. A follow up MRI demonstrating a new T2 and/or GAD enhancing lesion (in comparison to a baseline scan, with no specific time lag) or a single scan demonstrating asymptomatic GAD enhancing and non-enhancing lesions, are taken to be evidence of dissemination in time (DIT).

A diagnosis of primary progressive MS can be established if there is evidence of : (i) disease progression for at least one year and (ii) at least two of the following: (a) DIS (Brain) based on one or more T2 lesions in at least two characteristic regions i.e. periventricular/juxtacortical /infratentorial (b) DIS (Spinal Cord) based on two or more T2 lesions and (c) evidence of unmatched oligoclonal bands / elevated IgG index in CSF.

For both relapse onset and progressive onset disease, in the scenario of a brainstem/spinal cord presentation, the symptomatic lesions are not included in determining the fulfillment of criteria. Further, for purposes of demonstrating DIS, GAD enhancement of lesions is not included as it used to be in the earlier 2001 and 2005 McDonald criteria.

Cortical lesions are not part of the criteria for MS until now. With the use of higher field strength scanning (Pitt et al. 2010; Wattjes et al. 2009) and utilization of sequences that improve CGM lesion detection (DIR, PSIR, MPRAGE) and study (MTR), inclusion of cortical abnormalities has potential to further improve the sensitivity and specificity of the criteria.CGM lesions occur very early in disease and this suggests that an early diagnosis can possibly be established based on detection and distribution of intracortical lesions, as has been reported using DIR (Filippi et al 2010b).

1.4 Anatomy of the cerebral cortex

Grey matter is the portion of the central nervous system that is composed of the nerve cell bodies, proximal portion of the processes and the surrounding neuroglia. It is so called due to the grey colour. White matter, in contrast, consists of the nerve fibres in neuroglia, and is white in colour, the colour being attributed to the presence of high lipid content in the myelin covering the myelinated nerves (Ceccarelli et al. 2012a; Snell 1997.; Gandhi & Weiner 2012).

Grey matter covering the cerebral hemispheres forms the cerebral cortex and number of neocortical neurons has been reported as 21.4 billion in females and 26.3 billion in males (Pelvig et al 2008). The organisation into folds or convolutions (gyri) and fissures (sulci) significantly increases the surface area of the cerebral cortex. The thickness of the cerebral cortex ranges from about 1.5 mm to 4.5 mm – being thin in the sulcal pit (fissure) and thick over the crown / crest of each gyrus. It is composed of grey matter (nerve cells, proximal fibres, neuroglia) and blood vessels.(Compston et al. 2006; Snell 1997a.)

The main types of cells that make up the cerebral cortex include pyramidal cells, stellate cells, fusiform cells, horizontal cells of Cajal and cells of Martinotti. (Snell 1997a) The distribution of fibres within the cortex is in two directions, perpendicular to each other. This could either be radial or tangential. The tangential fibres, includes fibres that run along the cortical ribbon, parallel to the cortical surface. The radial fibres, make an angle of 90 degrees (perpendicular) to the cortical surface.

- Table 1.2 Revised McDonald Criteria (2010) (Polman et al. 2011)

Clinical Features		**Additional requirements to establish a diagnosis**	
Attacks	**Clinical Evidence**	**DIS**	**DIT**
>= 2	e/o >=2 lesions OR e/o 1 lesion with historical evidence of a previous attack	None	
>= 2	1 lesion	>=1 T2 lesion in at least 2/4 MS-typical regions of the CNS* OR Await further clinical attack implicating a different CNS site	
1	>=2 lesions		Simultaneous presence of asymptomatic GAD+ and GAD- lesions at anytime OR A new T2 and/or GAD+ lesion on follow-up MRI irrespective of its timing with reference to a baseline scan OR Await a second clinical attack
1	1	>=1 T2 lesion in at least 2 of 4 MS-typical regions of the CNS* OR Await a second clinical attack implicating a different CNS site	Simultaneous presence of asymptomatic GAD+ and GAD- lesions at anytime OR A new T2 and/or GAD+ lesion(s) on follow-up MRI, irrespective of its timing with reference to a baseline scan OR Await a 2nd clinical attack

(e/o – objective clinical evidence of ; GAD + :GAD enhancing and GAD – is non-enhancing,*periventricular, juxtacortical, infratentorial and spinal cord)

The cerebral cortex is divided into layers depending on the types of cells and the density and arrangement of cells within the cortex. The subpial surface (adjacent to CSF) is the superficial plexiform layer. The layers from outwards to inwards are the plexiform layer , external granular layer, external pyramidal layer, internal granular layer, ganglionic layer (internal pyramidal layer) and the multiform layer (layer of polymorphic cells) (Figure 1.2)

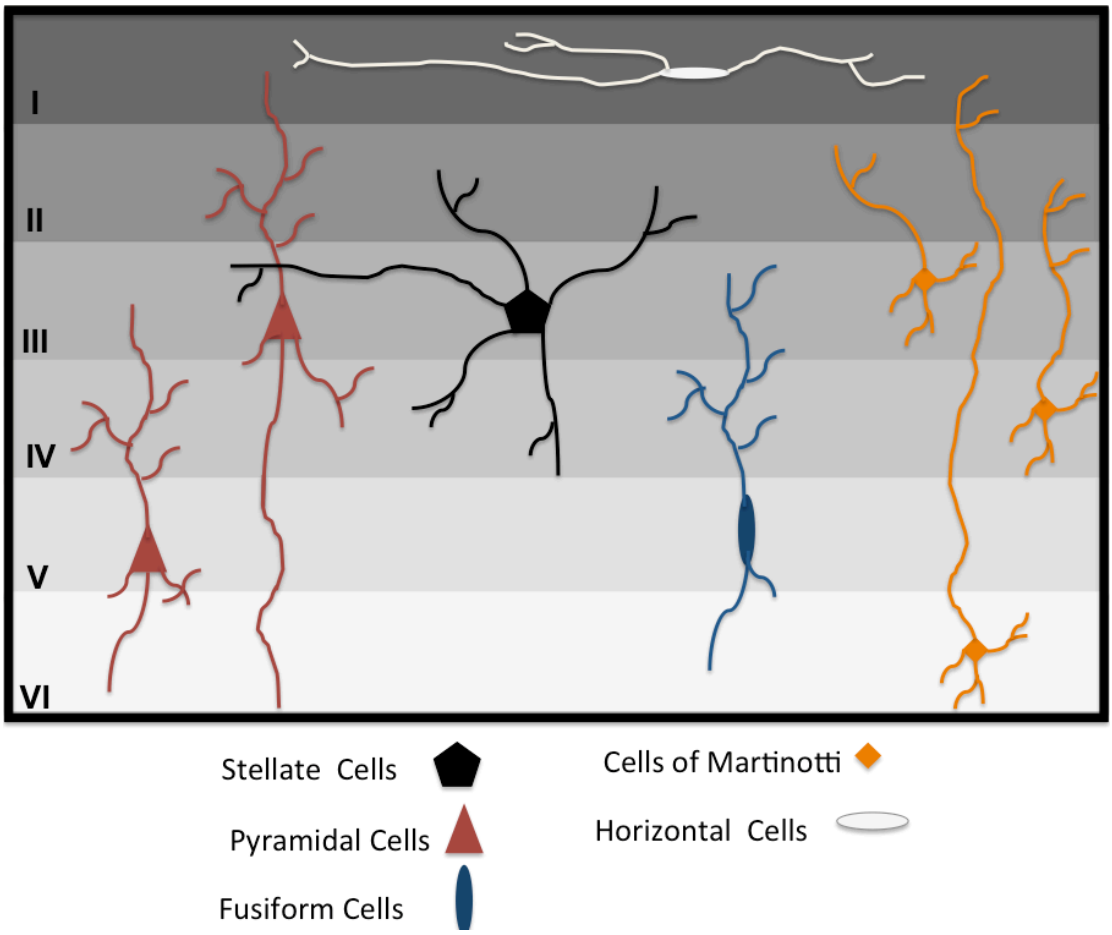
While most of the cortical surfaces are comprised of the these six layers, some regions where this clear arrangement cannot be demarcated are referred to as heterotypical regions. Examples of such regions include the post central gyrus, superior temporal gyrus and parts of hippocampal gyrus (all granular type) and other regions such as the precentral gyrus, other parts of the temporal lobe (agranular type).

It is important to appreciate this heterogeneity in the cortical structure to understand variations in imaging of the cortex – in health and in disease The functional specialisation of different regions has been extensively studied. However, given the complexity of the architecture of the cerebral cortex, the anatomical location is used to divide the cortex into lobes – the four main lobes being Frontal, Temporal, Parietal and Occipital. In addition some scientists further qualify the Insula and the limbic lobes as separate cortical regions.

Figure 1.2 : Layers of the cerebral cortex (Based on Snell 1997a)

I	**Molecular Layer**
II	**External Granular Layer**
III	**External Pyramidal Layer**
IV	**Internal Granular Layer**
V	**Ganglionic layer (Internal Pyramidal layer)**
VI	**Multiform layer (Layer of polymorphic cells)**

Figure 1.3 Types of cells in the grey matter (Adapted from Snell 1997a)



1.5 Current understanding of the pathophysiology of MS

In the section below I describe the current understanding of the pathophysiology of MS describing (i) pathological features of MS in grey and white matter and (ii) mechanisms thought to be responsible for these changes.

The 'lesion' or 'plaque' is the histological hallmark of the disease (Popescu & Lucchinetti 2012b) and represents multiple regions characterized by the loss of myelin. Demyelination is accompanied by inflammation, varying degrees of gliosis and preservation of axons(Popescu et al. 2013). Astrogliosis, injury to oligodendrocytes, degeneration of axons and simultaneous remyelination are other changes seen in lesions. Though initially thought to be limited to white matter, recent studies have established that demyelinating lesions are seen in the CGM as well. (Calabrese, Filippi, et al. 2010b; Geurts & Barkhof 2008; Peterson et al. 2001; Pirko et al. 2007) .

Pathological features

White matter lesions

White matter lesions continue to develop in patients with MS, even as older lesions evolve into chronic lesions, with no associated active inflammation. Acute, chronic and remyelinating lesions often co-exist in a patient. Different types of focal white matter lesions have been reported – these include classically active lesions, slowly expanding lesions and inactive lesions (See Figure 1.4)(Lassmann 2008b).

Acute active lesions are frequently seen in patients with relapsing remitting MS in the context of clinical attacks (Filippi et al. 2012). In acute lesions, there is a loss of the normal lamellar pattern of myelin, possibly as a

result of antibody related processes. Macroscopically acute white matter lesions are pink, soft and usually round or oval in shape. Microscopically the 'lesion' has distinct margins and a very cellular infiltrate consisting of lymphocytes, plasma cells, astrocytes and macrophages; myelin laden macrophages are the hallmark of an active lesion(Calabresi 2011). Preferential destruction of oligodendrocytes is seen in acute lesions (Sobel et al. 2008). There is significant oedema and minimal scarring noted in these lesions. Lesions are also noted to be distributed in the proximity of blood vessels i.e. perivascular.

Luchinetti et al. have demonstrated that acute lesions can be classified into four distinct patterns based on the heterogeneity of the immunopatterns. (Lucchinetti et al. 2000; Popescu et al. 2013).

Pattern I lesions are perivascular with sharp boundaries. Active demyelination is characteristically noted and there is no immunoglobulin deposition or complement activation. Pattern II lesions are also sharply demarcated lesions and in addition to evidence of active demyelination, are characterized by immunoglobulin and complement deposition and phagocytosis. In both Pattern I and II a variable loss of oligodendrocytes is noted at the lesion border. Remyelinating lesions show evidence of oligodendrocytes in the inactive centre of the lesions. Pattern III lesions show ill defined margins, oligodendrocyte apoptosis and demyelination with preferential loss of periaxonal myelin components. Immunoglobin deposition, complement activation and remyelinated lesions are not seen. The fourth immunopattern, Patter IV, is extremely rare and is characterised by a profound non-apoptotic death of oligodendrocytes in the perilesional WM.

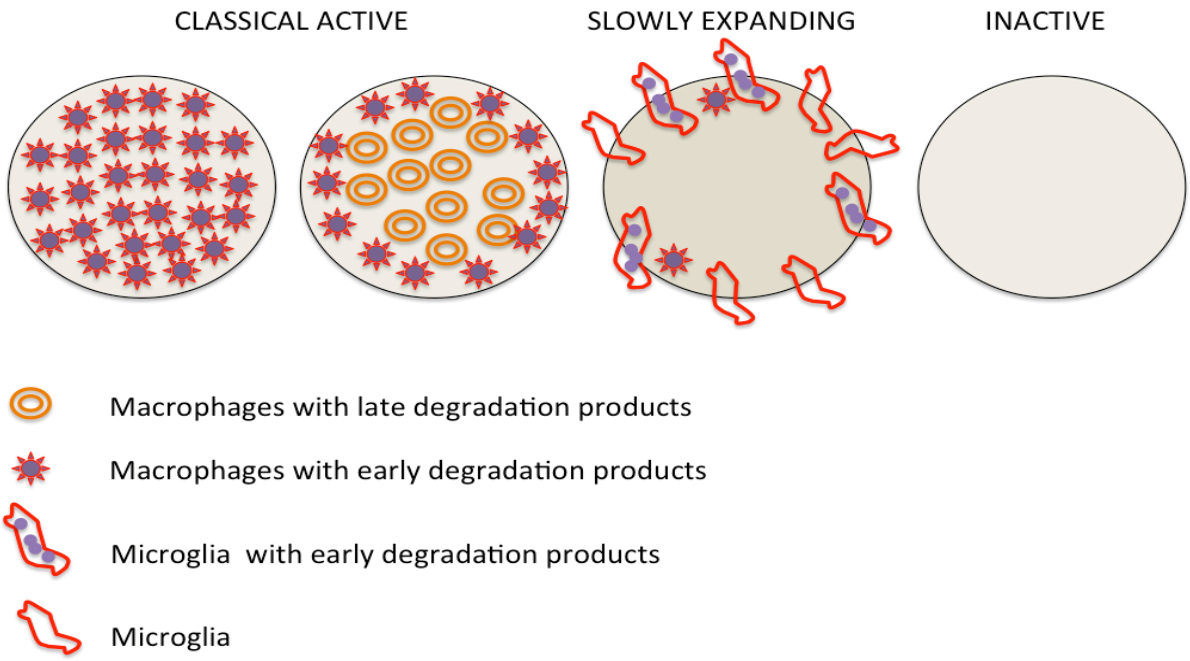
This classification of immunopatterns has been challenged in some studies (Barnett and Prineas et al. 2004). In a small series, Barnett and Prineas noted an overlap in the stages of complement containing macrophages and oligodendendrocyte apoptosis (an overlap between patterns II and III), and interpreted this to imply that the pathogenic heterogeneity of lesions depends on the age of individual lesions and not on the patient.

Variable axonal injury is seen as irregularly swollen axons, accumulation of amyloid B precursor protein and some degree of axonal loss. This is commonly seen in the context of acute lesions and is a factor responsible for the relapse related disability seen in patients. (Popescu & Lucchinetti 2012b; Filippi et al. 2012; Popescu et al. 2013). Retrograde degeneration has also been reported in the vicinity of the destructive JC WM lesions (Lassmann 2008b)

Slowly expanding lesions (SEL) differ from the classically active lesions. Macrophages containing all stages of myelin degradation products are rare and maybe even absent in SEL (Lassmann et al. 2007); a rim of activated microglia is characteristically seen in these lesions, and some of these microglia contain myelin degradation products. Perivascular infiltration of T cells is noted, but scarce. These smoldering plaques are thought to be responsible for progression (Popescu et al. 2013).

Figure 1.4 Different types of focal white matter lesions

(Adapted from Lassmann 2008b)



Chronic lesions on the other hand are firm and grey in their macroscopic appearance. The absence of inflammatory changes, and a greater extent of demyelination and axonal loss differentiates these from acute lesions. (Leary et al. 2009). Chronic lesions are more common in progressive disease. (Popescu et al. 2013) Chronic active lesions have a sharp edge and in addition to the macrophages and astrocytes, degenerating axons are noted in this phase, even as demyelination and remyelination continue to occur in parallel. Myelin in the form of droplets and a deposition of immunoglobulins differentiates these from acute lesions. In contrast to the increased cellularity in acute lesions, the centre of chronic lesions consists of demyelinated axons, with few lymphocytes and macrophages; no oligodendrocytes are seen. These chronic lesions also show increased astroglial scar tissue, thick vessels with hyalinised walls. (Lucchinetti et al. 1996; Allen et al. 2001)

Chronic inactive plaques are completely demyelinated, hypocellular lesions. They have sharp edges and are characterised by astrogliosis, loss of axons, absence of oligodendrocytes, minimal infiltration with macrophages, microglia and lymphocytes. As a lesion evolves from chronic active to chronic inactive, oedema and inflammation resolve and there is disappearance of macrophages and microglia. The demyelinated plaque then typically contains many glial fibres which are produced by astrocytes.

Remyelination of lesions, by cells of oligodendrocyte lineage (Bramow et al. 2010), can be seen in active MS patients. The degree and efficiency of remyelination is however less in patients with progressive disease (Bramow et al.2010; Lucchinetti et al. 2000). In these lesions thin myelinated axons with

short distances between nodes of Ranvier, are characteristically seen. They have a sharp margin and a reduced myelin density. When a lesion is completely remyelinated it is referred to as a “shadow plaque” These lesions are more vulnerable than the surrounding normal appearing white matter, to subsequent “attacks” (Prineas et al. 1993).

The normal appearing white matter (NAWM) shows diffuse inflammatory changes. Inflammatory infiltrates largely consisting of T lymphocytes , activated microglia and diffuse axonal injury are noted throughout the NAWM (Lassmann et al. 2007).

Grey matter lesions

Grey matter demyelinating lesions in MS have been reported in the cerebral CGM (Bo et al 2003; Vercellino et al 2005; Kutzelnigg et al 2005b). In addition the cerebellar cortex (Kutzelnigg et al 2007) and deep grey matter (Gilmore et al 2009; Vercellino et al. 2009) is also affected (Haider et al. 2014). Atrophy of deep grey matter nuclei, thalamus (Cifelli et al 2002) and caudate has been linked with the progression of clinical disease (Neema et al 2009). The changes observed in imaging studies are due to a combination of focal demyelinated lesions and diffuse changes in the normal appearing grey matter (Haider et al. 2014). Increased oxidative injury and anterograde or retrograde degeneration in iron rich regions of the brain have been postulated as a possible mechanism of these changes, and the association with clinical disability.

In the cerebellum, the extent of cortical demyelination is similar or even more than reported in regions of the forebrain in patients with MS (Kutzelnigg et al.2007). Demyelination with relative preservation of neurons,

axons and synapses, is seen in the context of cerebellar cortical lesions. In addition, scattered axonal swellings and end bulbs has also been reported (Kutzelnigg et al. 2007). The cerebellum also has an important role in cognitive functions and Cerasa et al (Cerasa et al. 2012) demonstrated that affection of neurofunctional networks responsible for communication between the cerebellum and cerebral cortex, impacts cognitive functions such as working memory.

In this project we focus on the study of grey matter lesions in the cerebral cortex and these are referred to as CGM. lesions. In comparison to WM lesions, CGM lesions have a smaller number of macrophages and lymphocytes and lesser amount of inflammation, microglial activation and astrogliosis. Cortical demyelination, neuronal loss and atrophy are seen in patients with MS (Bo et al. 2006a, Bo et al. 2003b).

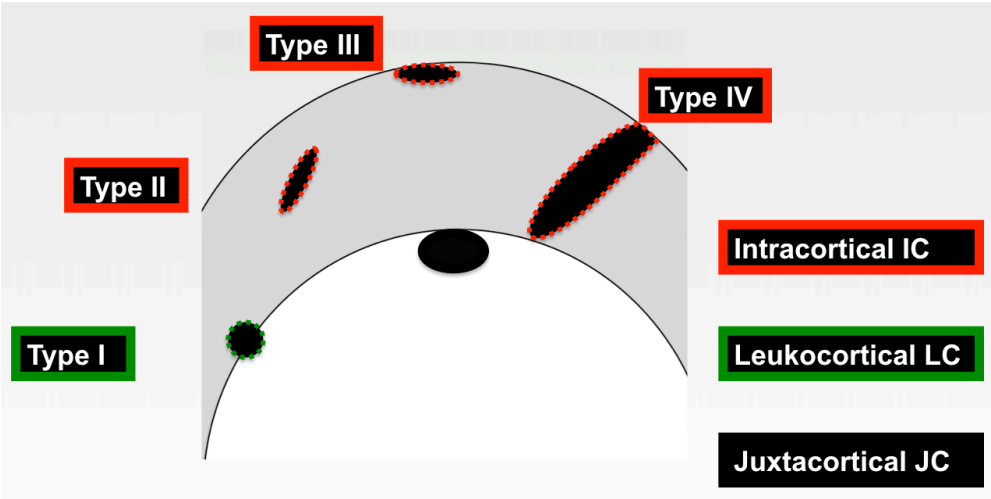
Based on their location within the cortex, demyelinating lesions have been classified into four types. (See Figure 1.5) (Bø et al. 2006a, Bø et al. 2003b, Wegner et al. 2009; Rudick et al. 2009). Type I lesions are at the border of the cortex and white matter, and involve both grey and white matter. Lesions limited to the substance of the CGM have been described as Type II lesions (Intracortical) and are noted to be perivascular in distribution. Type III lesions are seen in proximity to the meningeal surface, below the pia mater. They extend from the pial surface to cortical layer three / four or through the entire width of the cortex, becoming Type IV full thickness lesions.

Subpial lesions (Type III) dominate the histopathological findings in MS (Bø et al. 2003b; Albert et al. 2007b) and have been reported to constitute about 50 to 65% of the total cortical demyelination (Lucchinetti et al. 2011, Bø

et al. 2003b; Albert et al. 2007b). These are mainly seen in the sulcal folds, particularly in the insula, cingulate, frontal and temporal cortices, the hippocampus and the cerebellum (Lassmann et al. 2007, Lassmann 2008b).

Subpial lesions are seen as large band like lesions affecting the superficial layers of the cortex and can extend inwards from the outer surface of the cortex, and are oriented towards inflammatory infiltrates that are located in the leptomeninges. (Kutzelnigg et al. 2005a).

Figure 1.5 Classification of cortical grey matter lesions



(Types I – IV) and their corresponding radiological categories

The current view is that the neuropathological affection of the grey matter consists of (i) cortical demyelination with neuronal loss (diffuse) (ii) cortical atrophy(Lassmann 2008b) and (iii) synaptic changes including a change a loss of synapse and an impaired capacity of existing synapses (Wegner et al. 2006). The neuronal and axonal loss, in the early stages of disease, is seen on a backdrop of inflammation (Popescu et al. 2013). Pathophysiology of cortical MS lesions in general, is noted to have features that are quite different in comparison to white matter pathology. These include a scarcity of T and B cell infiltration, microgolial activation and astrogliosis. Remyelination is often seen in the CGM lesions and is more prominent than in the WM lesions (Albert et al. 2007b; Stadelmann et al. 2008).Blood brain barrier damage and edema is minimal / absent in the context of CGM lesions (Bø et al. 2003a). However, in meninges adjacent to CGM lesions extensive infiltration of T and B cells is seen. Meningeal inflammation (focal perivascular and diffuse) is prominent in early MS (Lucchinetti et al. 2011) and in some patients lymph follicle like inflammatory infiltrates are seen, specially in the patients with a more aggressive disease course. (Magliozzi et al. 2006).

Reduced density of neurons in the region of the CGM lesion and adjacent normal appearing cortex has also been reported. (Vercellino et al. 2005). In various studies, 10-36% neuronal and glial loss has also been seen in the cortex (Lassmann 2008b; Wegner et al. 2006; Vercellino et al. 2005) and is thought to be contributory to the reduction of cortical thickness seen in patients(Stadelmann et al. 2008); this is seen in both the demyelinated and the normal cortex (Wegner et al. 2006). Other features noted in CGM lesions include neuronal apoptosis, dystrophic axons and synaptic loss (Lassmann

2008b).

In early MS, most cortical lesions have myelin laden macrophages, indicating active demyelination. Inflammatory infiltrates of T cells, fewer B cells and minimal breakdown of the blood brain barrier (BBB) is present. In the more chronic cortical lesions, there is no breakdown of BBB and no evidence of inflammatory infiltrates or complement deposition. Microglia, apoptosis of neurons and damage to oligodendrocytes are also seen. In association with subpial lesions, profound inflammatory infiltrates of T cells, B cells and macrophages, have been described in late stages of disease. (Magliozzi et al. 2006). The degree of meningeal inflammation correlates with the degree of microglial activation, and in turn with the extent of demyelination, neurodegeneration and severity of clinical course. (Popescu et al. 2013)

Pathogenic mechanisms

Multiple sclerosis is considered to be an immune mediated disease with inflammation (Gandhi & Weiner 2012) against myelin, that is precipitated by environmental agents in predisposed individuals (Tsunoda & Fujinami 2002 , Frohman 2006) Demyelination with gliosis, inflammation and relative preservation of the axons is the traditional understanding of this pathology (Popescu & Lucchinetti 2012b). The variability and individual differences seen in the pathological features imply that there is probably no single or simple mechanism that can explain the pathogenesis of multiple sclerosis. (Lucchinetti et al. 1996; Lassmann & Lucchinetti 2008) The important features of pathogenesis in MS include (i) inflammation (ii) demyelination and (iii) injury of axons and neurons (Ellwardt et al. 2014; Gandhi & Weiner 2012; Bø et al.

2003b; Ceccarelli et al. 2012a; Compston 1999; Albert et al. 2007a; Dawson 1916)

The different mechanisms of tissue injury in multiple sclerosis lesions include (i) initial inflammatory tissue injury (ii) amplification of oxidative tissue injury (mitochondrial damage, iron liberation, retrograde degeneration) and (iii) astrocyte injury leading to an amplification of demyelination and neurodegeneration. (Lassmann 2008b)These mechanisms may all be seen in a lesion at the same time; however, their relative contribution is determined based on differences in lesion subtype, stage of disease and inter-individual differences. (Lassmann 2008a).

The inflammatory injury (related to active tissue injury) is seen across all stages of disease and involves both, the adaptive and innate immunity(Lassmann 2014) The degree of inflammation is more extensive in the early RRMS phase and reduces with the duration of disease (Frischer et al. 2009). Immune mediated mechanisms (Lassmann 2014) cause an active demyelination in relation to damage to the blood brain barrier (BBB), in the early stages of disease. Dendritic cells, monocytes, microglia, natural killer (NK) cells and mast cells are constituents of the innate immune system and they play a major role in the immune-pathogenesis of MS. Infections such as EBV affect the interplay between the T cells, cerebrovascular endothelium and adhesion molecules that recruit immune cells into the central nervous system.

A cascade of immunological events leads to demyelination (Frohman 2006; Geurts, Bø, et al. 2005a; Raine 1994). Different mechanisms including cytotoxic T cells, antibodies and production of reactive oxygen / NO

intermediates by activated microglia, contribute to this inflammatory injury. T- and B-cell immune mediated mechanisms represent adaptive immunity. (Frohman 2006; Trapp et al. 1998; Gandhi & Weiner 2012) and the response of the microglia is a manifestation of the innate immunity.

Following appropriate antigenic stimulation, CD4+ T cells differentiate into pathogenic myelin reactive CD4+ T cells. While the interaction with the antigen occurs in the periphery, a disruption in the blood-brain barrier allows these cells to enter the central nervous system and interact with the brain parenchyma and spinal cord, causing demyelination and as a result, acute MS lesions (Popescu et al. 2013). Regulatory CD24+ and CD25+ are deficient in MS patients; this is the principle to use one of the first line disease modifying drugs, glatiramer acetate which helps restore their numbers and build an immunological tolerance to self/auto antigens. (Viglietta et al. 2004; Frohman 2006)

Immune response in MS also involves the B Cell mediated immunity. B cells produce antigen specific antibodies, become the substrate for antigen presenting cells which are required for differentiation of T Cells and also produce a variety of cytokines. Patients with MS have an increase in the local, intra-thecal production of immunoglobulin's as demonstrated by an increased IgG index (Frohman 2006)and presence of oligoclonal bands in CSF (and not serum).

The use of newer techniques like microarray, has helped identify additional targets involved in the pathogenesis of a lesion e.g. alpha 4 integrin (alpha 4 beta1 and alpha4beta7) (Kleinschmidt-Demasters et al. 2012), a receptor expressed on T-cells aids with adhesion and transport pathways. A

specific monoclonal antibody against this receptor, natalizumab, (Frohman 2006; Rinaldi et al. 2011; Pucci et al. 2011) is an agent that has helped reduce the frequency of clinical relapses. It has also been shown to reduce the progression of disability and reduces the number of new T2 weighted and GAD enhancing T1-weighted lesions. Another similar target is osteopontin, which has been found to have an increased expression in MS lesions.

Antibody and complement associated mechanisms are responsible for demyelination and injury in the Pattern II lesions, the prime antigen target being the myelin oligodendrocyte glycoprotein. In pattern III lesions, the early changes are see in the sites most distal from the oligodendrocyte cell body, suggesting a dying back oligodendrogliopathy. The disturbance responsible for the appearance in pattern IV lesions is thought to be a primary metabolic and involves toxic action of the various inflammatory mediators.

In the later stages, there is an amplification of the oxidative tissue injury. Liberation of iron from oligodendrocytes (causing iron accumulation) and progressive mitochondrial damage lead to an expansion of WM lesions (pre-existing) (Lassmann 2014) and widespread diffuse injury to both the GM and WM. The accumulating damage to interconnected regions leads to retrograde degeneration of neurons. As a result, there is pre-activation of microglia, which on exposure to pro-inflammatory cytokines, differentiate into cytotoxic effector cells. As lesions progress, astrocytes within the lesions also become activated and transform into protoplasmatic astrocytes. This leads to a loss of molecules expressed by the distal astrocyte processes (aquaporin 4, excitatory amino acid transporter 2 and connexins). Excitotoxins thus accumulate in the extracellular space and there is loss of trophic support of

the oliogdendrocytes, axons and neurons. Under the effect of oxidative stress, the astrocytes become senescent and cause an increased production of pro-inflammatory cytokines. This results in increased demyelination and neurodegeneration.

Neuroinflammation and neurodegeneration are both recognized in MS(Ellwardt & Zipp 2014); however their temporal association is still not clearly understood. In the initial stages of RRMS, exacerbations are responsive to treatment with steroids, however in later stages of SPMS this is not seen, suggesting that neuroinflammation is not primarily responsible for the disability at this stage, which may in turn be due to a neurodegenerative process. Evidence of a degenerative process has however been seen even at disease onset(Ellwardt & Zipp 2014; Trapp & Stys 2009). Both innate and adaptive immunity contribute to the neurodegenerative process via effector molecules such as cytokines, glutamate or reactive species. The neurodegeneration in turn can be both primary and secondary. This is attributed to increased loss of axons, which maybe due to mechanisms that are related to demyelination or maybe demyelination independent (DeLuca et al. 2006). The axonal density reduces up to about 80% loss within the MS plaques, in patients with chronic disease. Until the point that up to 40% of axons are lost, no obvious physical disability may sometimes be apparent. Inflammation continues to be a parallel process, reaching minimal levels in chronic inactive lesions of long duration.(Popescu et al. 2013) As the disease progresses, the neurodegenerative mechanisms perhaps become independent of the inflammatory processes. (Deshmukh et al. 2013)

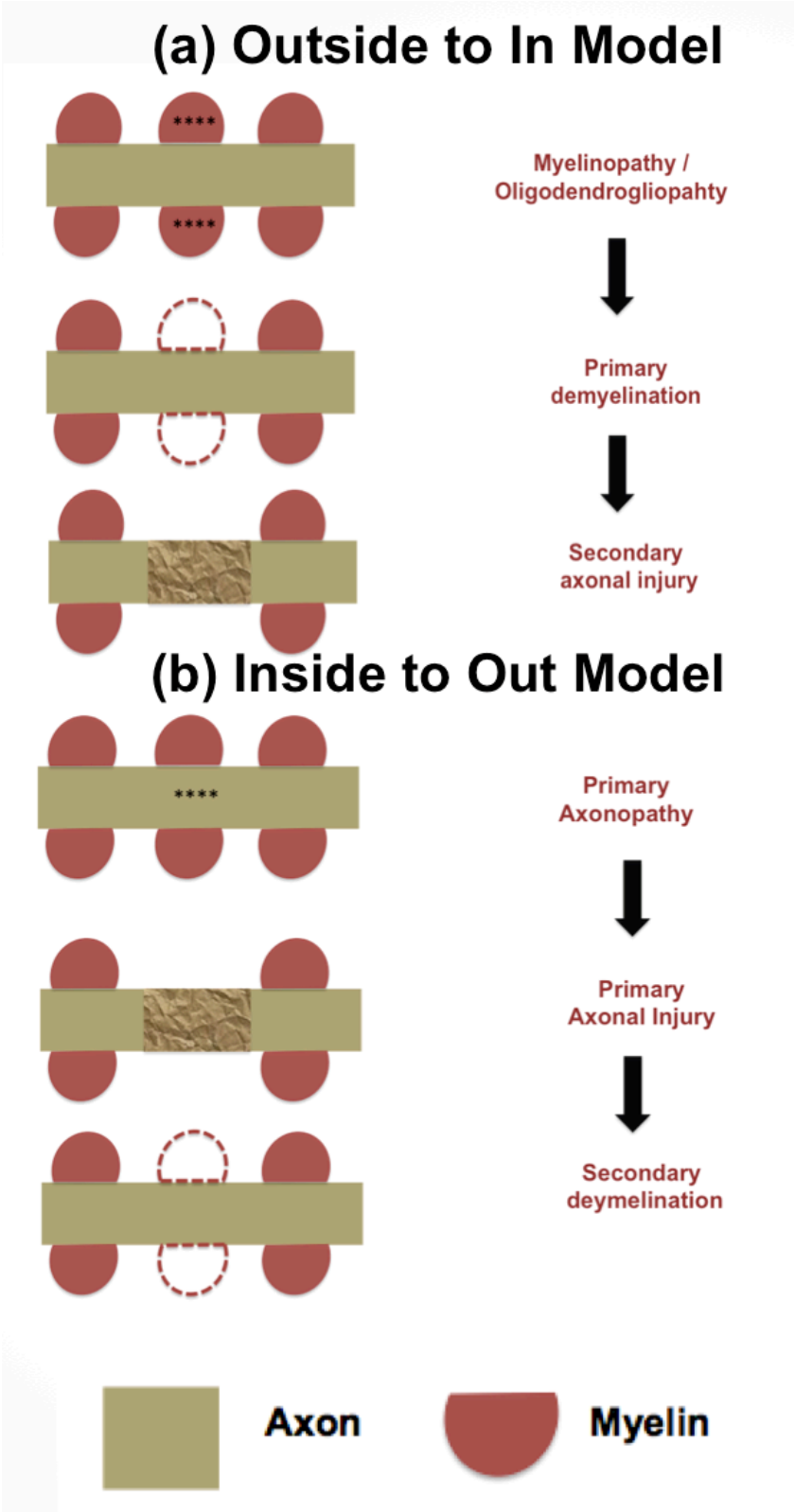
The primary target in the pathogenesis of MS, is thought to be myelin

(myelinopathy) or the myelin producing cell (oligodendrogliopathy)(Tsunoda & Fujinami 2002). Damage to the axons is thought to be secondary to the damage to myelin and the pathology moves from out (myelin) to in (axons) (Outside to In theory) (See Figure 1.6). The damage to the myelin be due to cytotoxic T cells and macrophages or toxic substances (cytokines, proteases). This however fails to explain the axonal damage seen in normal appearing white matter (Bjartmar et al. 2001)and the grey matter damage in the normal appearing grey matter(Sharma et al. 2001).

A contrary theory is the Inside (axon) to Out (myelin) model. This suggests that primary injury maybe axonal damage which in turn leads to demyelination. Mechanisms proposed include (i) disruption of crosstalk between the axon and oligodendrocyte (ii) induction of oligodendrocyte apoptosis by activated microglia and macrophages and (iii) triggering of autoimmunity by causing injury to the central nervous system. It is suggested that (Tsunoda & Fujinami 2002) both these models are not mutually exclusive and may act synergistically. In the region of the NAWM, secondary Wallerian degeneration contributes to the diffuse changes, however this alone cannot explain extent of injury seen (Lassmann et al. 2007). Diffuse axonal injury is at least partially independent of the changes in focal white matter lesions.

Figure 1.6 (a)Outside In and (b)Inside Out models of injury

(From Tsunoda & Fujinami 2002)



The pathogenesis of grey and white matter lesions may differ significantly and is still not clearly understood. Unlike white matter lesions, CGM lesions have minimal inflammatory changes and occur even in the absence of evidence for significant immune mediated damage. (Calabrese et al. 2011c; Nelson et al. 2007b; Geurts et al. 2011; Albert et al. 2007a; Traboulsee 2006; Calabrese 2007a; Bø et al. 2006b; Popescu et al. 2011,Vercellino et al. 2005)

Grey matter lesions

The mechanisms responsible for grey matter damage are broadly categorised as primary (developing inside the grey matter regions) or secondary (as a consequence of ongoing damage in the cerebral white matter)(Geurts & Barkhof 2008). It is suggested that both primary and secondary mechanisms are simultaneously responsible for the demyelination and axonal degeneration seen in the grey matter.

Secondary mechanisms of grey matter damage occur as a consequence of inflammatory demyelinating injury to the white matter. An abnormal and extensive distribution of sodium channels is noted in acute MS lesion following demyelination. These changes in expression of axonal sodium channels lead to an increased ATP demand. In addition, mitochondrial abnormalities are present due to being exposed to nitric oxide. This results in the mismatch of energy supply and demand, resulting in 'virtual hypoxia' (Stys 2004; Geurts and Barkhof 2008).

An increased concentration of metabotropic and ionotropic glutamate receptors and transporters is also found in the demyelinated and injured axons of the white matter and this glutamate dyshomeostasis has been

suggested as another secondary mechanism of grey matter damage. Upregulation of anti-apoptotic molecule bcl2 and ciliary neurotrophic growth factor signaling pathways is seen in the cortical neurons in MS patients and reflects a compensatory reaction to the demyelinating damage in the white matter; this can further lead to retrograde degeneration of the axons (Geurts and Barkhof 2008).

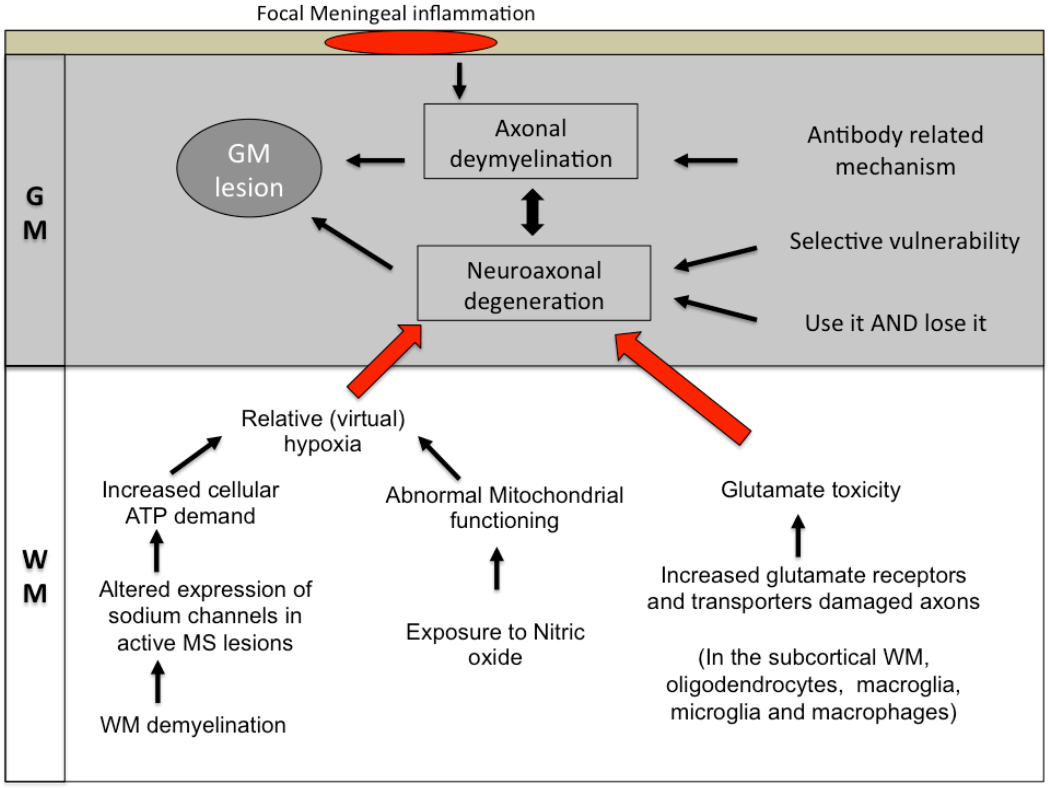
Primary mechanisms include (i) meningeal inflammation (ii) antibody mediated pathological mechanisms, which can be seen even in a complement independent manner (See Figure 1.7).

Meningeal inflammation (Magliozzi et al. 2007; Magliozzi et al. 2010) is noted from early stages of disease and can be both focal (perivascular) or diffuse, and is thought to contribute to the cortical demyelination and consequently even to the subcortical inflammation and demyelination in the white matter (Andersson et al. 1994, Popescu & Lucchinetti 2012a).

Ectopic B cell follicles have been noted in the leptomeninges of patients with MS. These changes follow a gradient in the superficial layers of the cortex, suggesting that cytotoxic or myelinotoxic factors from the CSF might play a role in the development of CGM lesions. In a cohort of 36 post mortem brain samples from progressive patients (29 SPMS; 7PPMS) studied by Magliozzi et al. (Magliozzi et al. 2007) ectopic follicles were found in 41.4% of the SPMS cases but not in PPMS. These follicles were mainly in the frontal, temporal and parietal lobes and in the cingulate gyrus and usually found in or at the entrance (rarely) of the cerebral sulci. Even in the follicle negative SPMS group, subpial demyelination was found to be more localized to the depth of the cerebral sulci. (Kutzelnigg & Lassmann 2006). The

preferential affection of subpial layers in the sulci led to the hypothesis that stagnant CSF mediators play a role in the development of CGM lesions (Stadelmann et al. 2008). By studying the distribution of lesions in vivo in regions in proximity to the meninges (sulcal pits; subpial lesions) (Serafini et al. 2004; Magliozzi et al. 2007; Magliozzi et al. 2010) this hypothesis can be tested.

Figure 1.7 Primary and secondary pathogenic mechanisms of GM damage (Adapted from Geurts & Barkhof 2008)



Complement deposition has not been observed in grey matter lesions (in contrast to white matter lesions). (Geurts and Barkhof 2008); the only exception being complement factor C4d seen in some MS cases. However antibody related pathological mechanisms leading to demyelination of cortical axons cannot be excluded. It is possible that (i) complement and auto-antibodies are present but their levels are below the threshold of detection by

current immunohistochemical techniques and (ii) antibody related damage is possibly even independent of complement (via Fc receptors on microglia, monocyte derived macrophages and natural killer cells (Brink BP et al. 2005).

Cortical regions that consistently show a high degree of metabolic activity, are believed to show a preferential degeneration in the context of ageing and Alzheimer's disease (Buckner et al 2005; Geurts et al. 2008). In MS a vulnerability of specific cortical regions (Sailer et al. 2003; Wegner et al 2003) such as the superior temporal gyrus, superior and middle fontal gyri has been noted; however factors contributing to this vulnerability remain to be clearly understood. Geurts et al (Geurts et al. 2008) suggest that further studies should explore if similar to neurodegenerative conditions like Alzheimer's, even in MS, regions of high metabolic activity are selectively vulnerable to damage – suggesting a 'use it and lose it' principle.

1.6 MR Physics

Magnetic resonance imaging (MRI), earlier referred to as nuclear magnetic resonance imaging, is a technique that allows in vivo study of the human anatomy. It uses the magnetic properties of hydrogen, when subjected to an external field and radiofrequency radiation. As part of water and lipids, hydrogen ions make up about 75-80% of the human body. A hydrogen nucleus contains a single proton. A proton is a charged particle which constantly spins around its own axis. As a proton has a mass, a positive charge and a spin, it produces a small magnetic field. The directional magnetic field in association with charged particles in motion is called *moment*. In the absence of an external magnetic field these spins are orientated randomly such that the net magnetic field within a subject or tissue

is near zero (McRobbie et al. 2003).

When subjected to the external magnetic field of the scanner (B0), the spins of the hydrogen nuclei align themselves in the direction of B0 so that there is a net longitudinal magnetisation vector parallel with B0. When another external field is then applied(RF pulse; B1) there is a shift from this equilibrium, resulting in a change in the net longitudinal magnetisation, which depends on the length and amplitude of B1. The net effect of the RF pulse is a flip of the net magnetisation vector, from being parallel to B0, to being shifted some angle away from it, creating a transverse component to the magenetisation.

Although the effect of B0 is to align the net magnetisation with it, the individual spins precess around B0. This precession causes the magnetic moments to follow a circular path, called the precession path, around B0 and the frequency of the precession is referred to as the precession frequency (Westbrook et al. 2011). The precessional frequency (w0) is determined using the Larmor equation and thus also called the Larmor frequency. As per the Larmor equation, the Larmor frequency is the product of strength of B0 and they gyromagnetic ratio. The gryomagnetic ratio is an intrinsic property of the spin species; for protons, this is ~ 42.6 MHz/T @1T so at 3T would be approximately 128MHz. The protons gyrating around the axis (precession) at the larmor frequency, in phase with each other, generate a rotating transverse magnetic vector.

Perturbing the magnetisation means that there will be a component of it perpendicular to B0. This perpendicular component of the magnetisation is measured and the changes as it returns to alignment with B0 is studied. The

pattern / nature of the perpendicular component can be changed by changing the B1. By altering the change to the net magnetisation that will be generated, the imaging sequences can be used to provide information such as the contrast between tissues.

When the external field is removed, the hydrogen nuclei release energy into the surrounding lattice (spin–lattice integration) and the net longitudinal magnetisation returns to being parallel with B0; this process is referred to as T1 relaxation or recovery. The spins also dephases and this depends on i) spin-spin interactions and ii) inhomogeneities in the external magnetic field; this process is referred to as T2 decay. The T2 of NAWM and a WM lesion at 1.5 T field strength, are ~61 and ~107 respectively; at 7T the T2 is ~25 for NAWM and ~55 for white matter lesions. The T1 relaxation and T2 decay times depend upon the properties of the individual tissues. When the effect of inhomogeneities in the external field are not accounted for, a shorter T2 decay is obtained, referred to as T2* decay. To adjust for these inhomogeneities spin echo methods are used which use 180 degree pulses which reverse the dephasing due to inhomogeneities. For any given tissue, T2 is always shorter than T1. Another important property of tissues that is utilised for generating images, is the PD of a tissue; this refers to the number of hydrogen atoms in a particular volume.

Pulse sequences are of two main types – spin and gradient echo. A spin echo uses two RF pulses to create an echo; following the administration of the excitatory RF pulse, the signal is refocused by a further RF pulse. The time between administration of the excitatory pulse and acquisition of the echo (peak of echo) is referred to as the echo time (TE). After the initial

excitatory RF pulse, it can be repeated; the time between the two excitatory pulses is referred to as the repetition time (TR). While good quality images can be created using SE, they are time consuming.

A gradient echo sequence consists of a pair of bipolar gradient pulses. There is no refocusing 180 degree pulse. Data are sampled during a gradient echo; this gradient echo is achieved by dephasing the spins with a negatively pulsed gradient before they are rephrased by an opposite gradient with opposite polarity.

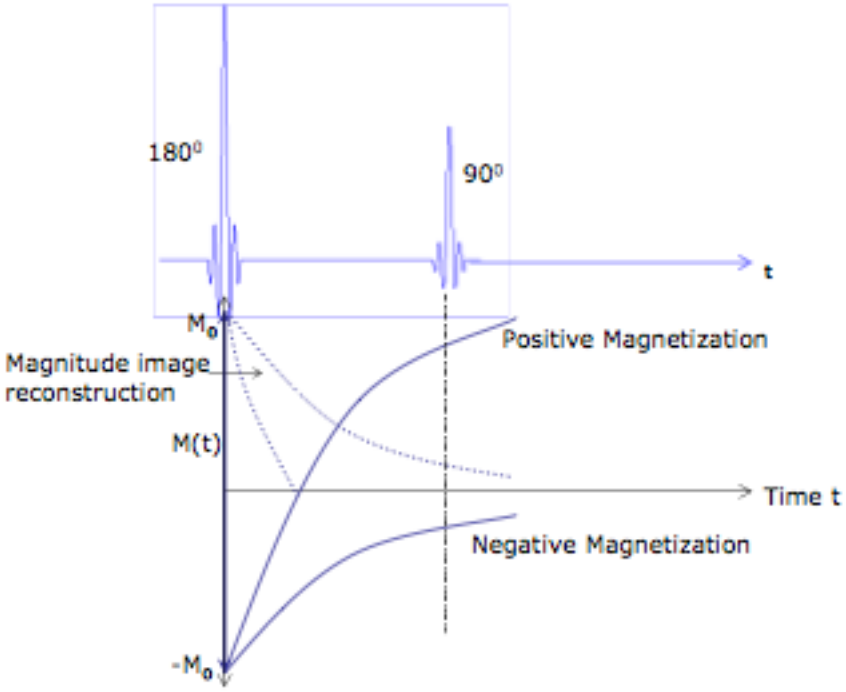
In order to produce images with predictable contrast between tissues, the images are 'weighted' preferentially towards T1 / T2 or PD. This is achieved by altering the TE, TR and the initial flip angle caused by the excitatory pulse (for gradient echo sequences) (McRobbie et al. 2003).When both TR and TE are short (TR <750ms and TE < 40ms), a T1w image is generated (i.e. the image contrast is governed by differences in the T1 of the tissues) and when both are long (TR> 1000ms TE>75ms) a T2w image is generated. For PD-weighted image, the TE has to be short and the TR has to be long.

Phase sensitive inversion recovery

An Inversion recovery (IR) sequence is a T1w MRI sequence that is able to generate a contrast between different tissues based on the differences in the T1 relaxation times of various tissues. It is a variation of the spin echo sequence (McRobbie et al. 2003) in which a 180 degree RF pulse is used to invert the longitudinal magnetisation prior to the 90 degree excitation pulse. The parameters that specify a particular IR sequence are the inversion time (TI), repetition time (TR) and the echo time (TE).

IR sequences are often used to suppress fluid and fat. Variants of this sequence are created by either adjusting the imaging parameters or adding additional pulses. These include short tau inversion recovery (STIR), fluid attenuated inversion recovery (FLAIR), spectral presaturation inversion recovery (SPIR) and phase sensitive inversion recovery (PSIR). Inversion recovery sequences have also been used for cardiac imaging to detect myocardial infarctions; estimation of pulmonary blood flow and perfusion; detection of hemorrhage in atherosclerotic plaques etc.

Figure 1.8 Generation of PSIR sequence (From Garach et al. 2004)



The IR image can have values ranging from –M0 to +M0; where M0 is the tissue magnetisation at thermal equilibrium. As the image contains negative values, the real part of the complex image needs to be reconstructed so as to benefit from the complete contrast range. The IR image has

information pertaining to both magnitude and polarity, which are stored in the magnitude and phase component of the complex image respectively.

The phase component that stores information regarding polarity is referred to as the intrinsic phase. An additional component of phase is the background phase, which is due to factors such as errors in acquisition, field inhomogeneities and off-resonance phenomenon (McVeigh et al. 1986; Garach et al 2004).

In conventional IR reconstruction, only the magnitude images is used, so as to overcome the undesirable effects due to the background phase (Garach et al. 2004). In PSIR, in addition to the magnitude, the intrinsic phase information is also used, giving the full dynamic range (-M0 to M0), providing a greater T1 contrast (Zhou et al. 2004).

For purposes of PSIR reconstruction, polarities (intrinsic phase) associated with each image pixel needs to be determined. The intrinsic phase can be estimated by subtracting the background phase from the total phase. To estimate the background phase, multiple acquisitions (with different TI) can be used, however this increases chances of errors due to problems with registration, motion artefacts etc. An alternative approach is using algorithms based on the differing characteristics of intrinsic and background phase (Borrello et al. 1990; Garach et al. 2004; Ma et al. 2005).

A Markov Random field (MRF) model has been successfully used for estimating the background phase (Garach et al. 2004). The background phase of two neighboring pixels can be determined by comparing the phase difference between two adjacent pixels, with and without adding 180 degrees to the phase of one of the pixels. A combination that provides the minimum

phase difference is selected to be the background phase and the optimum combination can be determined by analyzing all possibilities between adjacent pixels. To do so for the whole image is not computationally practical. To overcome this two different approaches are used (i) block merging and (ii) region growing. (Garach et al. 2004).

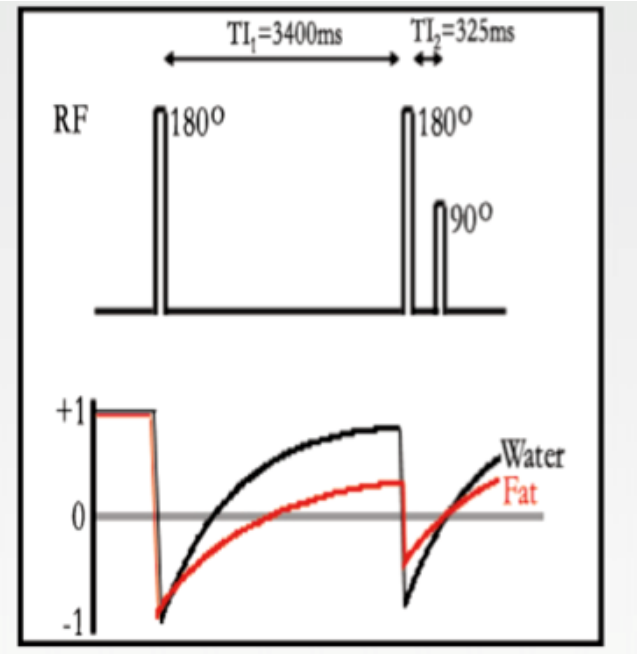
In block merging (also called “divide-and-conquer” method), the phase map is divided into many 2x2 blocks and each such block is optimized. The next step involves combining neighboring blocks into 4x4 blocks, 8 x 8 blocks, 16x16 blocks etc. until the entire field of view is combined into a single block. Each sub-block then differs in phase from the adjacent blocks. Flipping the phase within sub-blocks and creation of representative pixels (mean of pixels along the row) along each row are additional steps to achieve the best possible optimization and background phase can then be estimated. Using this information, the PSIR image can the be reconstructed on a pixel by pixel basis. This multiple resolution procedure is helpful given the slow varying nature of background phase.

An alternative approach to achieve optimization is the region growing method which is helpful in conditions of high variations in the background phase (Garach et al. 2004). This starts from a single point and then ascertains the optimal set of parameters along a spiral path; thus starting from a small number of pixels and expanding till the entire field of view is covered. The four centre pixels of the image are the first to be optimized; following this, the ‘region growing’ starts along the top left corner. Following a circular trajectory, moving from the top left to the top right corner, and then to the bottom right and bottom left corner, returning back to the top left corner. At

this point, the process would then move to the top left corner of the next larger loop. This method of optimization is specially useful for situations with a low SNR image in the presence of high variations in background phase.

Over the past few years, another new sequence, double inversion recovery (DIR) has been extensively used (Figure 1.9) and has improved detection of cortical lesions. In this sequence, the CSF and white matter signals are suppressed, leading to improved visualization of the grey matter and CGM lesions.

Figure 1.9: Generation of the Double inversion recovery sequence



(Adapted from Boulby et al 2004)

1.7 MRI of MS lesions

Magnetic resonance imaging (MRI) is a non-invasive method to achieve high-resolution images of the brain and spinal cord. It has significantly changed the study and management of MS since first being used in the 1970s (Compston et al. 2006).

Imaging of White Matter in MS

The ability to readily detect focal white matter abnormalities in patients with MS, enabled magnetic resonance imaging to become an important aid in the diagnosis and management of this condition. (Filippi et al. 2011).T2-weighted, Post-contrast T1 weighted and Fluid Attenuated Inversion Recovery sequences are the most established conventionally used protocols for studying MS. A 'lesion' appears hyper-intense on a T2w/FLAIR sequence, the histopathological correlate being inflammation or edema/ abnormal myelination/ gliosis or axonal loss. (Filippi et al. 2011). On T1w spin echo sequences, 20-30% lesions appear hypointense, and are commonly referred to as 'black holes'. The intensity can however be variable, tending towards either the lighter GM or the darker CSF; reflecting different degrees of pathological severity. While most acute black holes are seen to resolve / disappear over a duration of about six months, about 36% persist; these persistent black holes represent regions of low myelination (Schmierer et al. 2004). Acute inflammatory lesions (up to 4 weeks), show enhancement on T1w sequences, when contrast agents like GAD are administered. This is because of the breakdown of the blood-brain barrier seen in acute lesions. Some of these GAD enhancing lesions evolve into being persistent black holes. (Filippi et al. 2011; van Waesberghe et al. 1998)

White matter lesions in MS have been described to have characteristic shapes and distributions. Lesions in the brain may be ovoid in shape, and commonly seen in the periventricular (including corpus callosum), juxtacortical and infratentorial regions. The periventricular lesions tend to be perpendicular to the surface of the ventricles commonly referred to as 'Dawson's fingers'.

Distribution is often variable and asymmetric and the lesion load does not correlate with the stage/ severity of disease (Fisniku et al. 2008). Patients with PPMS have been noted to have a generally smaller number of brain lesions when compared with relapse onset MS. (Lucchinetti et al. 2004).

In the spinal cord, lesions may appear cigar shaped on sagittal scans. Their width usually encompasses less than half of the diameter of the cord, and the length is limited to less than 2 vertebral bodies. A predilection for the cervical cord, and for the posterior and lateral columns has been noted across phenotypes. Typically, these lesions do not show any mass effect, however may do in the acute phase.

In addition to the lesion load, brain atrophy, quantified using T1w images has been another frequently used measure of disease and is found to be a better indicator of prognosis (Miller et al. 2002). The atrophy noted in MS can be due to destructive processes like demyelination and axonal loss and can also be the result of resolution of oedema in acute lesions. The onset of neurodegenerative processes may also contribute to this in the later / progressive forms of disease. Brain atrophy is increasingly used as an endpoint / measure in clinical trials focused on neuroprotection (Barkhof et al. 2009b).

Imaging of Grey matter in MS

The goals of grey matter imaging research are to quantify the disease burden, help in diagnosis, predict disability and guide treatment; together with being able to understand the kinetics of disease progression. In addition to CGM lesions, grey matter atrophy, diffuse grey matter damage and abnormalities in deep grey matter have been reported using MRI (Ceccarelli

et al. 2012). Grey matter atrophy is a sensitive marker for neurodegeneration and can be detected even in early disease. MS phenotypes have been differentiated based on the distribution of regional grey matter atrophy, which has also helped predict the extent of cognitive impairment and symptoms of fatigue (Ceccarelli et al. 2008).

Diffuse abnormalities in grey matter have been shown in studies using Diffusion tensor imaging (DTI) (Calabrese et al. 2011c) and Magnetisation Transfer Ratio (MTR) (Tur et al. 2011; Davies et al. 2004; Fox et al. 2005). Techniques such as susceptibility weighted imaging (SWI) (X. Chen et al. 2012; Hagemeier et al. 2012; Ropele et al. 2011) have also helped to understand the role of iron deposition in MS. These show changes in early phases of disease and can be monitored over time or provide evidence to support /refute effects of iron deposition (SWI) and explore whether it might be a cause / effect of grey matter lesions. (Stankiewicz et al. 2007; X. Chen et al. 2012). Abnormalities in the deep grey matter (Geurts et al. 2008) including abnormal metabolite concentrations, T2 hypointensities in the basal ganglia and thalamic atrophy and neurodegeneration (Cifelli et al. 2002) have been reported.

Conventional magnetic resonance imaging (MRI) sequences such as T2 weighted sequences and Fluid attenuated inversion recovery (FLAIR), are very sensitive for detecting white matter lesions but are less sensitive in detecting CGM lesions. CGM lesions are not seen well on conventional T2w image because of the prolonged relaxation times of the grey matter which in turn results in a poor contrast between the lesional grey matter and the surrounding tissue. (Kidd et al. 1999) In comparison to WM lesions, CGM

lesions have a higher cellular density with a greater number of neurons and a lesser degree of inflammatory infiltrate and this is responsible for the increase in relaxation time. Other reasons that make the visualisation of CGM lesions difficult include the effects of partial volume due to the CSF. This particularly interferes with the detection of subpial pathology which is reported to be about 60% of the cortical demyelination (Bø et al. 2003b). Imaging of CGM lesions is also limited by their small size and the minimal disruption of the blood brain barrier (which thus precludes the use of contrast agents like gadolinium to help increase identification)(Bø et al. 2009; Seewann et al. 2011).

Other difficulties in the study of CGM lesions include the interference of white matter lesions in the development of automatic algorithms to segment the grey and white matter (Gandhi & Weiner 2012; Chard et al. 2010; Frohman 2006; Trapp et al. 1998). New techniques for overcoming this problem are being developed, including methods of lesion-filling which helps to minimize errors in the estimation of global and regional grey matter volume. (Ceccarelli et al. 2012b; Gelineau-Morel et al. 2011). In addition to CGM lesions, grey matter atrophy is a potentially more sensitive indicator of neurodegenerative processes; it increases over time and is more resistant to changes due to fluid volume fluctuations (Bø et al 2003b; Pirko et al. 2007) and imaging techniques that help with this will help improve our understanding of disease progression.

While the ability to use stronger field strengths has improved GM lesion detection (1.5Tesla, 3Tesla),(Ceccarelli et al. 2012a) newer sequences

that allow visualization of different aspects of pathology have further enhanced this. DIR is one such sequence. It allows the classification of CGM as being intracortical (IC; corresponding to type II and IV) or leucocortical (LC; corresponding to Type I). (Geurts, Bø, et al. 2005a; Geurts & Barkhof 2008). Subpial (Type III) lesions however are not visualised. As the name suggests, DIR allows for a 'double' suppression of both CSF and white matter. In comparison to FLAIR, DIR has improved the detection of grey matter lesions by almost 2.6 times. (Calabrese et al. 2007; Seewann et al. 2012; Calabrese et al. 2010c; Rinaldi et al. 2010) Strong correlations have also been found between the grey matter lesion load (number and volume) and the markers of clinical disability (EDSS) and cognitive impairment tests in some studies (Calabrese 2010) and the results need to be reproduced by other centers. Consensus recommendations to help identify GM lesions on DIR were recently published based on a consensus of raters from six different centres. (Geurts 2011) CGM lesions have been shown in patients with early stages of disease (clinically isolated syndrome) (Filippi 2010b), and even in pediatric patients with MS. (Absinta et al. 2011; Giorgio et al. 2011; Ceccarelli et al. 2012a)

The increased use of imaging in the clinical setting today has often led to the detection of MRI findings which are suggestive of MS, in patients with no symptoms /signs of MS. This condition is referred to as the Radiologically isolated syndrome. It is believed that RIS is a pre-clinical / subclinical form of MS; with up to 2/3 of patients showing radiological progression and 1/3 developing clinical evidence of MS. (Giorgio et al. 2011; Granberg et al. 2012) CGM lesions have been reported even in this subset of patients

(Granberg et al. 2012; Giorgio et al. 2011), and the number and distribution of CGM lesions could potentially predict the progression to disease in these patients.

However, more recent post-mortem work has suggested that DIR identifies only about 18% of the lesions seen prospectively with imaging. Histopathological and radiological comparison found that DIR has a high specificity but a low sensitivity to CGM lesions (Seewann et al. 2012); even after complying with the international consensus recommendations for marking lesions.(Gandhi & Weiner 2012; Trapp & Nave 2008; Bø et al. 2003b; Geurts et al. 2011) In addition, it has not been possible to classify the lesions to mirror histopathological subtypes, being potentially incapable of quantifying whether or not subpial lesions that dominate histopathological findings, are in fact seen using imaging.(Gandhi & Weiner 2012; Ceccarelli, Bakshi, et al. 2012a).

Other limitations of DIR include low SNR. The variable intensity of the image and the artefacts due to blood flow and partial volume effects of CSF further add to its limitations. In an attempt to overcome these problems and bridge the histopathological - radiological gap, newer sequences are being studied with the goal being to increase in-vivo detection of CGM lesions. Phase sensitive inversion recovery is one of the techniques that appears promising for improving detection of CGM lesions (Goris 2012; Nelson et al. 2007a). It has also been suggested that DIR and PSIR be used in conjunction(Frohman 2006; Trapp 2008; Crawford 2004; Filippi 2010b; Zang et al. 2004; Gandhi & Weiner 2012; Nelson 2007b)

1.8 Cognitive impairment in MS

In patients with MS, the prevalence of cognitive impairment (CI) ranges from 40 to 70%. Different aspects of MS pathology (lesions, NAWM, GM atrophy) involving both the cortex and the connecting WM tracts are relevant in determining CI (Rao et al. 1991).

Factors responsible for cognitive impairment in patients with MS include: (i) damage to the WM tracts, which is thought to contribute to impaired cognitive functions that rely on rapid transfer of information i.e. attention, Information processing and Executive function and (ii) abnormalities in the cortex such as focal cortical lesions , atrophy and abnormal cortical integrity (Jongen et al. 2012).

The patterns of cognitive impairment seen in MS commonly affect domains of attention / processing speed; learning /memory and executive function. In addition episodic memory – requiring repetition or recall of serially presented verbal or visual information, is also affected in patients with MS (Filippi et al. 2010a; Rao et al. 1991).

These problems however are rarely picked up as these (i) are rarely a presenting symptom; (ii) are not picked up on the brief mental status examination done on the bedside; (iii) require assessment of a variety of functions to be able to identify dysfunction; (Lezak et al. 2012) and also (iv) tend to be overlooked in patients where physical disability is a prominent deficit (Rao et al. 1991)

The association between T2 lesion load and neuropsychology is modest, allowing us to believe that following damage to the white matter, there is a functional disconnection between cortical and deep grey matter

structures. While the cerebral cortex has been recognised as a highly organised “seat” of control, the relation between a cortical location of lesions and an effect on behaviour / cognition can still not be confidently predicted. (Lezak et al. 2012)

The interpretation of location – behaviour correlations is made difficult as the same function can involve different regions of the brain, and in turn each cortical location may be responsible for many tasks. At both the microscopic cellular levels, and also at the level of macroscopically definable cortical zones, there is a large degree of overlap and integration. The multitude of neuropsychological assessments are only able to measure the final effect on a particular behaviour or function, which may in turn comprise multiple tasks that occur in parallel.

Widely used test batteries used in MS include the (i) Rao Brief Repeatable Neuropsychological Battery (BRNB) (Strober et al. 2009), which requires less time and can be translated into many European languages; (ii) Minimal Assessment of Cognitive function in MS (MACFIMS) (Dusankove et al. 2012),which has a stronger psychometric foundation and includes assessment of spatial processing and higher executive function abilities. This can consist of either of the Symbol Digit Modalities Test (SDMT) (Benedict et al. 2012) – which takes only 5 minutes to complete and has a slightly better ability to predict the diagnosis, disease course and work disability - (or) the Paced Auditory Serial Addition Task (PASAT) (Polman and Rudick 2010).

Speed dependent tasks and tasks requiring the transfer of information across cerebral hemispheres, unmask an impaired processing speed in patients with MS. (Archibald & Fisk 2000) This is more obvious with tasks that

require auditory verbal stimuli vs. those requiring visual stimuli. (Lezak et al. 2012). As the extent of disease progresses, the performance in these tasks progressively deteriorates and is apparent on less demanding tasks as well. (Grant et al. 1984) It is also noted that alternating attention and divided attention are often impaired in MS and subtle deficits can be unmasked by using tasks that require patients to alternate between stimuli or perform tasks simultaneously (Archibald and Fisk 2000).Various tasks are used to demonstrate impairment in information processing speed. (Lynch et al. 2010) These include word fluency tests, symbol digit tests, the stroop colour word interference task and the PASAT. All these are designed so as to require the subject to process serial data rapidly, with the usual goal being to complete the maximum number of items in a given period of time. Impairment is often seen irrespective of disease status in those tasks that require inhibition of previously given correct responses.

PASAT is the only cognitive test that is routinely a part of the MS functional composite score (Fischer et al. 1999). A series of 61 single digits are presented to the subject as an auditory stimulus, at a fixed rate. The task requires the subject to give the sum of each new number, and the one preceding it. The typical interval between tasks is 3 seconds. While considered to be a gold standard by some, this test has limitations. Often the participants skip alternate numbers (skipping strategy) and give a good performance. The effects of education, age and practices effects are noted as confounders. It is not a popular test among patients who often find this rather distressing. The task does not selectively assess processing speed and invokes other cognitive processes like working memory, complex attention

and mathematical skills. (Lynch et al. 2010). Poor performance on PASAT in MS patients is suggestive of slow processing speed rather than problems with working memory.

The tests of cognitive function carried out in this project (Hayling, Stroop, immediate and delayed story and figure recall, PASAT and SDMT) were chosen based on their frequent use in clinical neuropsychology assessments. They form a comprehensive assessments of the domains that are known to be affected in people with MS – memory (Story and figure recall), information processing (SDMT and PASAT) and executive function (Hayling and Stroop). These tests are routinely used to assess cognitive function in people with MS within the Queen Square Hospital. An alternative approach would have been to use the Brief Repeatable Neuropsycholgical Battery, which is also a widely used measure of cognitive function in MS studies (Strober et al. 2009, Achiron et al. 2003). However this battery would not be as sensitive as the tests I used. In addition, the tests used e.g. Stroop, are also widely used in other neurological conditions, not just MS, so it is easier to assess comparable effects (such as damage to specific networks) and gain converging evidence from other conditions.

1.9 Summary

MS is a neuro-inflammatory condition that affects both the white and grey matter. The understanding of grey matter pathology in MS has improved in recent times. This is due to improved methods of histopathological identification of lesions and improved radiological methods that allow in vivo detection of pathology. Conventional MRI using T2-weighted, or FLAIR sequences, reveals few CGM lesions, and is considerably less sensitive to

detection of CGM lesions. Double inversion recovery sequence is now accepted as an improved method of studying cortical pathology. However it is limited by artefacts and low signal to noise ratio. A T1w inversion recovery sequence has recently been developed, which improves the contrast between grey and white matter. This phase sensitive inversion recovery has a higher SNR and has the potential to improve the study of cortical pathology.

Grey matter pathology in the form of demyelinating lesions and atrophy is present across all phenotypes of MS and is seen even in early stages like in CIS. Recent histopathology studies have demonstrated more extensive GM demyelination in people with progressive compared with relapsing-remitting (RR) MS, suggesting a potential role in the accrual of irreversible disability. In addition, CGM demyelination has been linked with meningeal inflammation, providing a plausible mechanism for this to occur independently of WM demyelination. However, it is not known how closely GM and WM lesion accrual is linked in life, and whether or not the relationship differs between RR and progressive forms of MS. This is important, as current disease modifying treatments for MS have been proven to reduce WM lesion accrual, but it cannot be assumed that they are equally effective against GM demyelination, particularly if the two processes are not closely related.

Addressing this question with histopathological studies is difficult as tissue is usually only available from people with early atypical clinical presentations or at post mortem (i.e. those with clinically advanced MS) and examination of the whole brain is prohibitively time consuming. Similarly, addressing this question in vivo with magnetic resonance imaging (MRI) has been hampered by very limited GM lesion detection using conventional

scanning methods. Using MRI techniques such as double inversion recovery and more recently phase sensitive inversion recovery (PSIR), it is now possible to do so.

In this project detection of CGM lesions on DIR was compared with a higher resolution (PSIR) sequence, to explore if it can help improve the study of CGM lesions. This work investigated the anatomical accuracy of PSIR versus DIR in detecting and localising CGM lesions respectively. A representative cohort, including patients with RR, PP and SPMS, was recruited in this project, together with controls in order to allow a comparison of findings with those healthy subjects who do not have MS. I aim to suggest possible guidelines for the detection of grey matter lesions using PSIR, highlighting the areas of artefacts / false positives. A study of the intra-rater and inter-rater reproducibility will help ascertain the ability of this sequence to be used across different raters and centres.

Given the more detailed anatomical visualization on PSIR, I hypothesize that PSIR will improve the detection of CGM lesions, both quantitatively and qualitatively. Correlations of CGM lesion number and volume with cognitive function and physical disability measures has been studied using DIR. (Giorgio & De Stefano 2010; Jongen et al. 2012; Calabrese et al. 2011a; Calabrese et al. 2009) Cognitive impairment has been shown to correlate with CGM lesion load and I expect a similar correlation to be seen with lesion load on PSIR. Using the sequence, I recorded the distribution of CGM lesions within the cortex and across the cortical lobes; and studied the hypothesis that the distribution of lesions impacts on the pattern of cognitive impairment seen in patients. Patients with

RR and SPMS and some healthy controls, were also invited to take part in a follow up arm of this study. The follow up study was undertaken to understand how CGM lesions evolve with time.

In order to investigate the specificity of PSIR-detected CGM lesions, I compared the findings of CGM lesion pathology in MS patients with patients diagnosed with other conditions that have white matter lesions. By comparing these findings with healthy controls and patients with another neurological disease (Fabry's disease) I explored how detection of CGM lesions might help in differential diagnosis. Two main questions are the (i) significance of CGM lesions – with respect to clinical and cognitive impairment and whether the location and distribution of these lesions can aid in differentiating patients with MS from other neurological conditions that have white matter lesions. (ii) Secondly, from a mechanistic point of view, can the distribution, location and evolution of lesions help improve the understanding of the pathogenesis of cortical pathology and delineate possible pathogenic mechanisms in the evolution of grey matter lesions.

In vivo detection of CGM lesions has the potential to improve the understanding of pathogenesis and also guide diagnosis and management. Histopathological correlations with lesions, their classification using both DIR and PSIR and detailed analysis of topographical distribution seen with both these scans are future directions in the study of grey matter pathology in MS.

2 Improved detection of cortical grey matter lesions in MS using phase sensitive inversion recovery

2.1 Abstract

Objective

CGM lesions are common in multiple sclerosis (MS), but usually not seen on MRI. I compared the performance of double inversion recovery (DIR, currently considered the best available imaging sequence for detecting CGM lesions) with phase sensitive inversion recovery (PSIR, a sequence allowing much higher resolution scans to be obtained in a clinically feasible time).

Methods

Sixty MS patients and 30 healthy controls underwent MRI scanning on a 3 Tesla scanner. I compared intracortical (IC) and leucocortical (LC) lesion counts obtained with a standard DIR sequence (1x1x3mm resolution, obtained in 4 minutes) and a PSIR sequence (0.5x0.5x2mm, 11 minutes). Lesions were marked separately on DIR and PSIR scans.

Results

In the whole MS cohort, more CGM lesions were seen on the higher resolution PSIR than the DIR scans (IC mean±SD: 18.1±9.8 vs. 5.9±4.5, $p<0.001$; LC mean±SD: 13.4±12.9 vs. 7.3±8.0, $p<0.001$). On PSIR, ≥1 IC lesion was seen in 60/60 MS patients and 1/30 controls, and ≥1 LC lesion in 60/60 patients and 6/30 controls. On DIR, ≥1 IC lesion was seen in 50/60

patients and 0/30 controls, and ≥1 LC lesion(s) in 60/60 patients and 5/30 controls.

Conclusions

Compared with DIR, using PSIR I was able to detect a significantly greater number of CGM lesions. The presence of at least one IC lesion in every MS patient, but very few healthy controls, suggests that it may be a useful adjunct to conventional MRI when a diagnosis of MS is suspected but not confirmed

2.2 Introduction

Histopathological studies of grey matter pathology (Bo et al. 2001; Bo et al 2003a) are limited by the specimens available (mostly progressive patients with long duration disease) and by technological limitations (each slice of brain examined is not representative of the variability seen across brain parenchyma). In vivo detection using imaging has the advantage of providing data across all phenotypes of disease, even in patients with short duration of disease and can be repeated non-invasively. DIR is currently the modality of choice for detection of CGM lesions (Geurts et al. 2011). However as demonstrated in histopathology literature, it identifies only a small part of the total pathology. There is a need to improve radiological detection of GM lesions. PSIR has been developed as a sequence to aid CGM lesion detection (Hou et al. 2005).

The aim of this work is to investigate the use of the phase sensitive inversion recovery sequence versus double inversion recovery sequence, when used independently. I shall examine the correlations between lesion number estimated, using both DIR and PSIR, and scores of clinical and cognitive performance. It is hypothesised that grey matter pathology is responsible for poor performance on cognitive tests and by studying these correlations I will explore this relationship, and also study whether DIR and PSIR, help understand the contribution of cortical pathology to cognitive impairment.

Given the improved resolution of PSIR, I hypothesised that there would be an improvement of CGM lesion detection in comparison to DIR. If in

spite of the improved SNR and grey/white contrast with PSIR, this is not seen, this could be possibly because (i) CGM lesions have been over-estimated using DIR or (ii) a different subset of lesions is identified using PSIR. Similarly, if correlation between cognitive tests and CGM lesion load is not seen, this could be due to a differential lesion detection in the two sequences / an overestimation of the true lesion load on DIR.

2.3 Methods

2.3.1 Participants

A cohort of 60 patients with MS and 30 controls, all in the age group of 18 to 65 years, were recruited for this study. This included 15 patients each with primary and secondary progressive disease(Lublin & Reingold 1996) and 30 patients with relapsing remitting MS. Informed consent was taken from all participants. The study was approved by the local ethics committee.

2.3.2 Data Collection

All participants underwent scanning with a 3T Achieva TX system (Philips) using a 32 channel head coil. A detailed history and neurological examination was also recorded. Expanded disability status scale (EDSS(Kurtzke 1983)) was estimated and the Multiple Sclerosis Functional Composite(MSFC)(and its components- 9 hole peg test (9HPT), 25 foot timed walk 25TWT and PASAT Paced auditory serial addition test) (Polman & Rudick 2010) and Symbol Digit Modality Test (SDMT)conducted (Benedict et al. 2012) .

Table 2.1 Demographics of study participants

MS Subtype	N	Age in years (mean ±SD, range)	Sex M: F	EDSS (median, range)	Disease duration in years (mean±SD, range)	Number who received DMTs
PP	15	51.9±9.1 (34-65)	5:10	6 (1.5-6.5)	12.1±8.0	3
SP	15	50.9±7.6 (36-64)	7:8	6.5 (4.5-8.5)	23.3±7.3	11
RR	30	42.5±9.6 (25-64)	10:20	1.75 (1-6.5)	11.5±10.5	23
All	60	47±9.9 (25-65)	22:60	4.75 (1-8.5)	14.6±10.4	37
Controls	30	37.8±11.7(22-64)	12:18	-	-	-

(PP=primary progressive; SP=secondary progressive; RR=relapsing remitting MS; DMD=disease modifying drugs)

Table 2.2 Acquisition Parameters

	Resolution (mm)	FOV (mm2)	TR (ms)	TE (ms)	TI (ms)	Slices	SENSE	Time (mins)
T2	1x1x3	240 x180	3500	19/85	-	50	1.7	4.01
FLAIR	1x1x3	240 x180	8000	125	2400	50	1.3	3.4
DIR	1x1x3*	240 x180	16000	9.9	3400/ 325	50	2	4.16
PSIR	0.5x0.5x2	240 x180	7306	13	400	75	-	11.26

(FOV=field of view, TR=repetition time, TE=echo time, TI=inversion time, SENSE=sensitivity encoding factor.

*In one MS patient only, the DIR was acquired at 1x1x2mm)

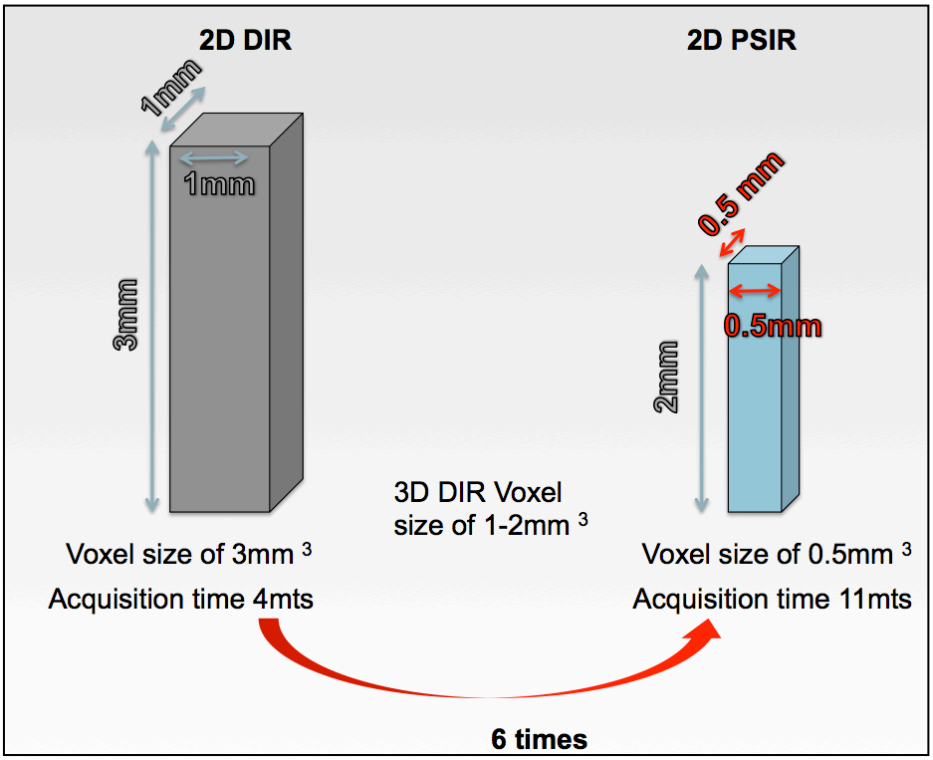
2.3.3 Images Acquired

Conventional images including PD and T2-weighted images were acquired, together with FLAIR, DIR and PSIR. The acquisition parameters used are given in Table 2.2. The resolution using PSIR was increased about six times (Figure 2.1).

Each voxel on DIR (Figure 2.1) had an in-plane resolution of 1mm and a slice thickness of 3mm, giving it a voxel size of 3 mm^3(Calabrese et al. 2007; Simon et al. 2010). This was acquired in a scan time of 4.16 minutes. On PSIR, the in-plane resolution was 0.5mm, and a slice thickness of 2mm, giving it a voxel size of 0.5 mm^3 and this was acquired in 11.26 minutes. Thus a much higher resolution (6 times) was obtained in a clinically acceptable time of eleven minutes.

Image sequences such as 3D DIR have been studied at higher field strengths and provide a resolution with a voxel size of 1-2 mm^3 (Seewann et al. 2012; de Graaf et al. 2012) While it is theoretically possible, to get sub-millimeter voxels with DIR, this would lead to a prohibitively long scan time. In addition to the time taken to get the same resolution as PSIR, if 0.5x0.5x2mm scans are acquired using DIR, there will be a 27% loss in the SNR and in order to prevent this loss, the scan time would be further increased. The scan time is inversely proportional to the square root of SNR and thus the scan times would go up about six times.

Figure 2.1 Comparison of DIR and PSIR voxel size



2.3.4 Image Analysis

JIM (version 6, Xinapse Systems) was used to mark and annotate lesions. Images were marked on DIR and PSIR independently. I marked lesions under supervision of two senior raters. I was blinded to the demographics of the patient. In order to prevent a bias, enough time was ensured between marking of the DIR and PSIR scans to prevent the memory of markings from influencing subsequent markings.

CGM lesions were marked in the cerebral and cerebellar cortex – data for supra and infratentorial lesions was recorded separately. For marking lesions on DIR, the published recommendations (Geurts et al. 2011) were used as a guide: a lesion was considered to be hyper-intense with respect to the surrounding cortex. In addition each identified lesion was at least 3 pixels in size and could be seen on contiguous slices. Symmetrically located hyperintensities were likely to be artefacts. Regions of the insula, temporal poles, occipital vertices were identified as artefact prone regions and were marked with caution.

As there were no published guidelines available for marking on PSIR, I established a set of rules was in consultation with an expert senior rater (Tarek Yousry, Professor of Neuroradiology). These rules are summarised in Table 2.3 below.

Guidelines on marking grey matter lesions on PSIR

These guidelines apply to lesions to be marked on PSIR images acquired using a 3T scanner, with a resolution of 0.5mm x 0.5mm x 2mm. On PSIR, a lesion is hypointense with respect to the surrounding cortex, and a corresponding hyperintensity on FLAIR and presence of lesion on contiguous

slices are features used to confirm lesions. A lesion that involves the CGM, even though if mostly in the white matter is considered a CGM lesion. If lesions appear to merge into one , they are counted as one lesion. To avoid marking false positive lesions PSIR slice is to be reviewed with corresponding FLAIR image when in doubt. For posterior fossa lesions, comparison should be made with corresponding PDT2 slices. This comparison helps confirm lesions and also helps reduce false positives due to vessels, CSF (especially in sulci), and artefacts.

If small or faint, a lesion is to be accepted only if visible on 2 or more consecutive slices. Thin and linear hypointensities (more likely to be vessels) are to be avoided; some cortical lesions have a linear appearance and should be marked carefully using above criteria. Lesions that involved more than one slice were counted on the slice where they were most obvious. When marking lesions it is also important to keep a constant magnification and contrast settings. The insula, temporal poles were identified as regions prone to artefacts. VR Spaces may falsely give the impression of being lesions especially in the basal ganglia – lentiform nuclei, subcortical white matter of the temporal lobes

Classification of lesions

CGM lesions were classified based on their involvement of grey and white matter. An intracortical lesion is limited to the thickness of the grey matter; and is a pure CGM lesion. This could theoretically correspond to Types II, III or IV in the histopathological classification described in Chapter 1. Lesions that involved the grey matter, but also involved the white matter, were classified as mixed i.e. leucocortical lesions, corresponding to Type I in the

histopathological classification. Examples of these lesion types are shown in the Figure 2.2 and 2.3. The definition of the leucocortical lesion is also clearer on PSIR.

A third type of lesion that could be identified on PSIR included the juxtacortical lesion. This is a white matter lesion, abutting but not involving the grey matter. It was identified by observing the preservation of the normal contour of the cortex and maintenance of the grey-white border. On DIR, these lesions could not be separated from the leucocortical lesions. See Figure 2.4

Morphology of Lesions

While counting lesions, it was noted that lesions which had a curvilinear morphology were increasingly frequent amongst the patients group. The number of these lesions was also counted separately. The other shapes noted included oval and wedge shaped lesions. All these shapes have been described in earlier work using DIR. An example of a curvilinear shaped lesion is shown in corresponding PSIR and DIR images is shown in Figure 2.5

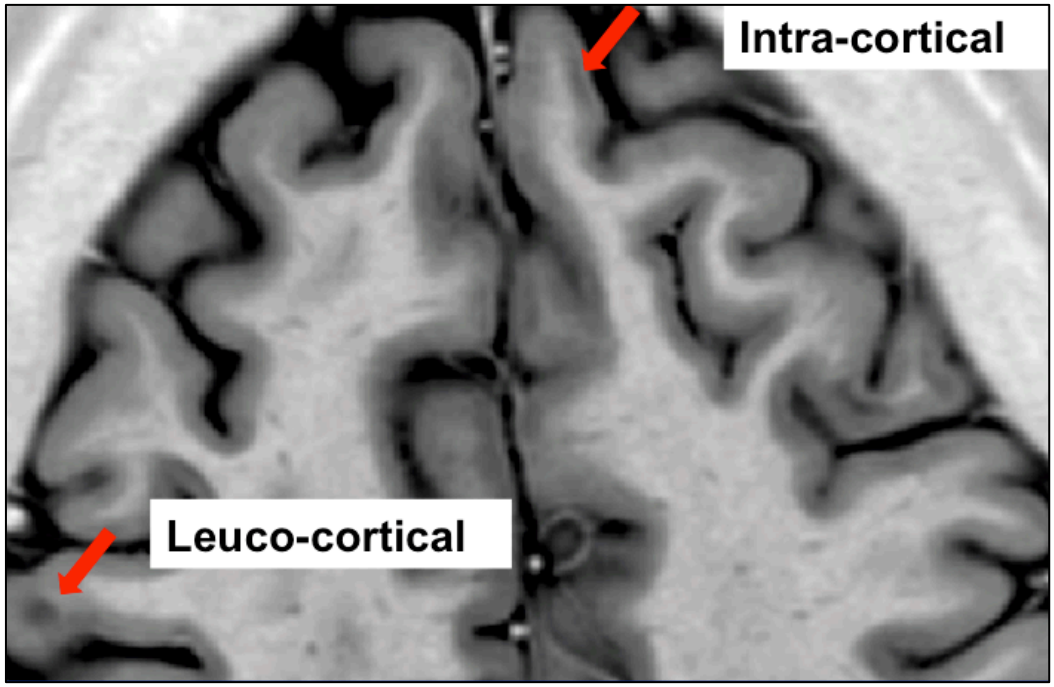
Artefacts

As on DIR, artefacts could be seen on PSIR. However these were fewer compared to DIR. These included Virchow Robin spaces, especially in the region of the insula and the temporal poles. As shown in the figure 2.6, these are perivascular spaces but as they appear as hypointensities on PSIR, they could be mistaken for lesions. Furthermore they do not have a consistent appearance on FLAIR and this can thus not be used to help identify these as artefacts.

Table 2.3: Rules for marking cortical grey matter lesions on PSIR

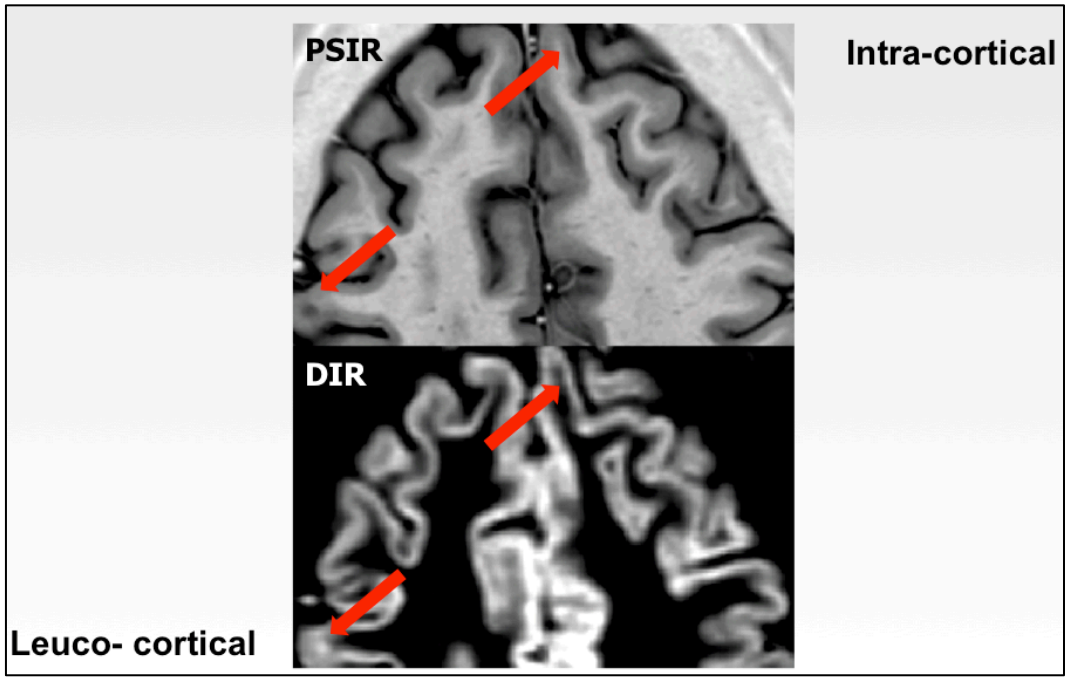
Inclusion	Lesions are hypointense relative to surrounding normal cortex
	They must involve the cortex in part or whole a. A lesion confined to the cortex is called Intracortical (IC) b. A lesion that involves both cortex and juxtacortical white matter is called leucocortical (LC) (See Figure 2.2,Examples of IC (top right) and LC (below left) lesions Figure 2.3,Figure 2.4)
Caution	If small or ill-defined on a single slice, they must be visible on at least one other contiguous slice
	Equivocal lesions must be confirmed by retrospective detection of signal abnormality compatible with a lesion at the same location on the corresponding T2-weighted and/or FLAIR images
Exclusion	*Adjacent cerebrospinal fluid (partial volume effect)*, determined after reviewing adjacent PSIR slices and corresponding T2-weighted and FLAIR scans; especially likely in sulcal regions, at the temporal poles and near the vertex
	Artefacts, sometimes recognized by their symmetrical appearance (as also seen in DIR images)
	Vessels, which form very thin linear hypo-intensities, especially those that do not follow the direction of the cortical ribbon (cortical lesions may have a curvilinear or linear appearance, but they are less thin and invariably follow the direction of the cortical ribbon (See Figure 2.7)
	Virchow-Robin spaces, which are prone to manifest in certain cortical regions (in particular the insula and temporal poles), especially if linear and multiple, forming mesh-like or bundle-like appearances (these were seen in patients and controls (See Figure 2.6).

Figure 2.2 : Examples of types of CGM lesions



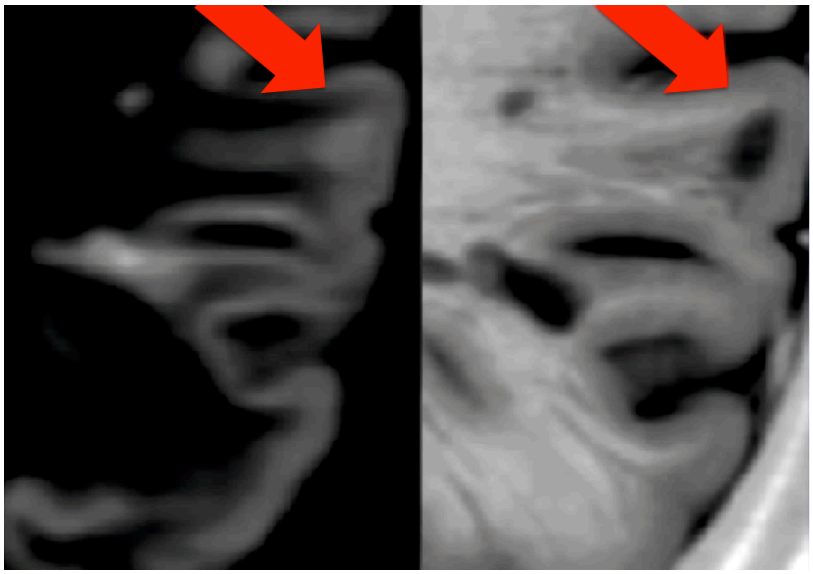
Examples of IC (top right) and LC (below left) lesions

Figure 2.3 Comparative DIR and PSIR images



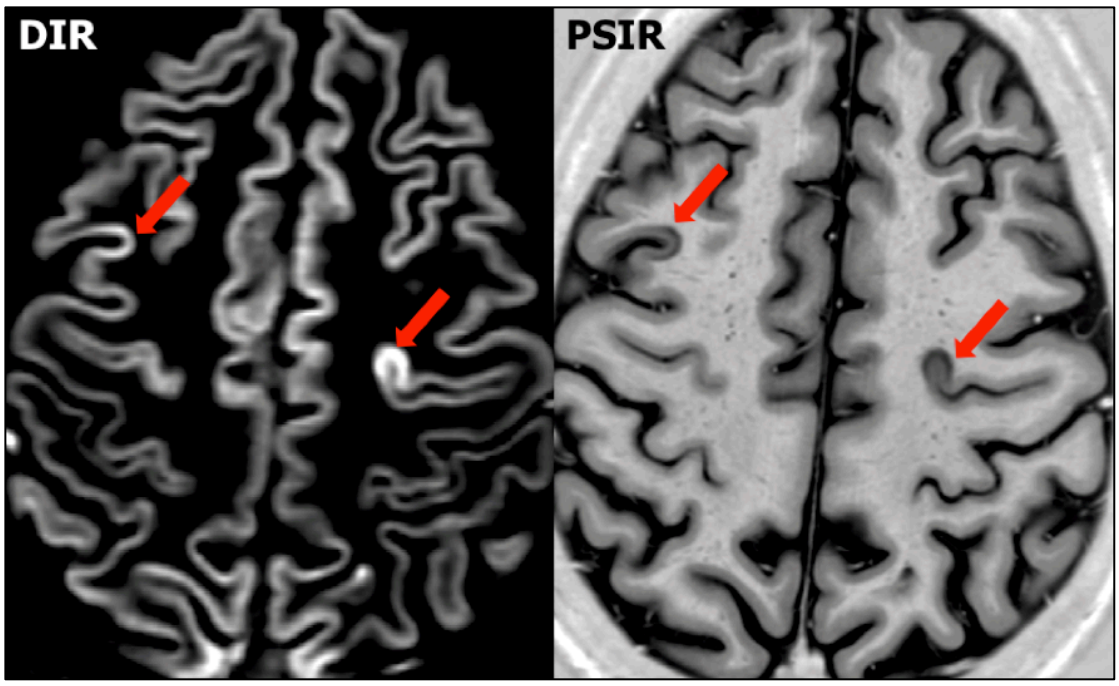
Examples of IC and LC lesions as seen on DIR and PSIR

Figure 2.4 Example of a juxtacortical lesion



Note : Appears LC on DIR, but can be seen to be limited to WM on PSIR

Figure 2.5 Curvilinear morphology of lesion shown on DIR and PSIR



It was however noted, that unlike lesions, which seem to be uniformly hypointense, Virchow-Robin spaces had a mesh like appearance; this feature could sometimes be used to help identify them. An example is shown in Figure 2.6 Another common artefact seen on PSIR, included blood vessels (Figure 2.7) These also appear as linear hypointensities and thus can be mistaken to be a lesion. Looking at contiguous slices helps: a blood vessel will tend to travel with the slices, unlike a lesion, which would tend to remain localized to the same spot.

Lesion Counting

CGM lesion numbers for all different lesion types were counted separately. This included intracortical and leucocortical lesions on both DIR and PSIR. In addition juxtacortical lesions were counted on PSIR, but could not be counted on DIR. To assess non-juxtacortical white matter lesion loads, these were counted on PSIR and PD/T2 scans.

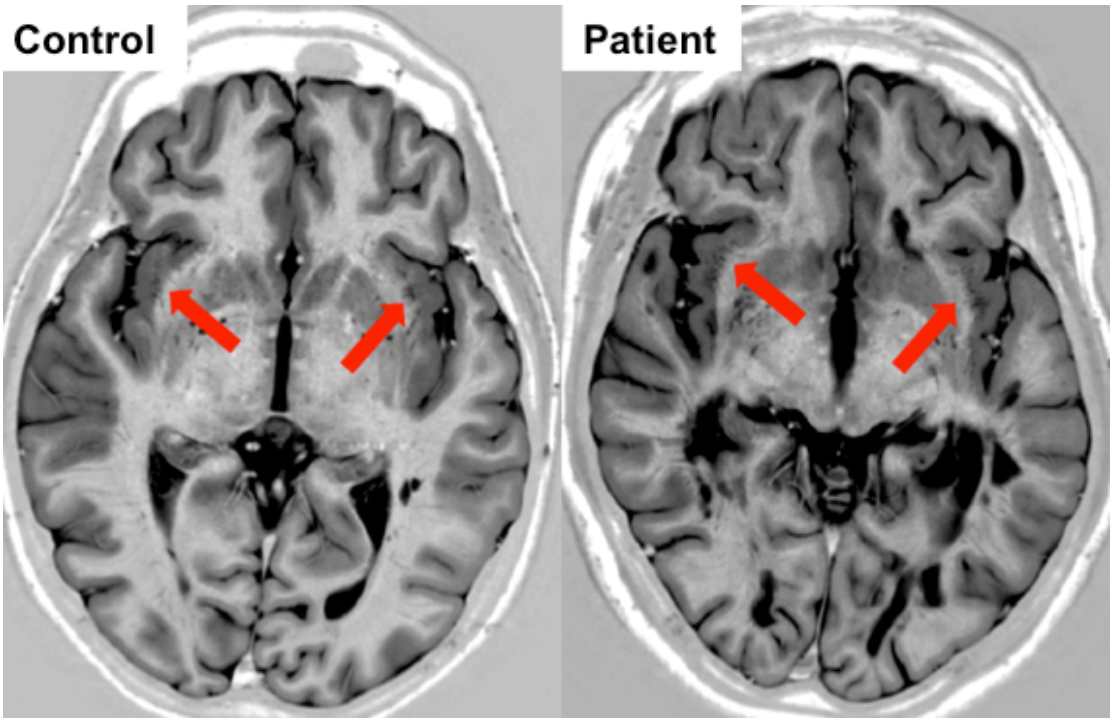
To assess inter-rater reproducibility of marking lesions on PSIR, 10 randomly selected scans were evaluated independently by three raters (Varun Sethi, Nils Muhlert and Declan Chard) using the guidelines described above. To assess intra-rater reproducibility, the scans of these 10 patients were marked three times by me. To assess the contrast-to-noise ratio between grey matter and white matter, 10 healthy controls were randomly selected. In each control, 12 regions of interest (ROI) were placed on three separate slices, in regions of normal-appearing CGM (at the level of vertex, insula and temporal poles) and neighbouring normal-appearing white matter. The ROI size was chosen to be large enough to sample grey matter signal, but small enough to be fully contained in the grey matter in each subject. The ROIs were placed in

the same location for each control on DIR and PSIR images. Mean absolute signal values were noted, and the relative contrast between grey matter and white matter on both sequences was calculated using the formula below, as described by Mainero et al. ($C_{gm\text{-}wm} = S_{gm}\_S_{wm}/V_{gm}$; where $C_{gm\text{-}wm}$ is the contrast between the grey matter and white matter; S_{gm} is the mean signal for the ROI placed in the grey matter; S_{wm} is mean signal for the ROI placed in the white matter; and V_{gm} is the variance in the signal intensity of the grey matter ROIs).

Statistical Methods

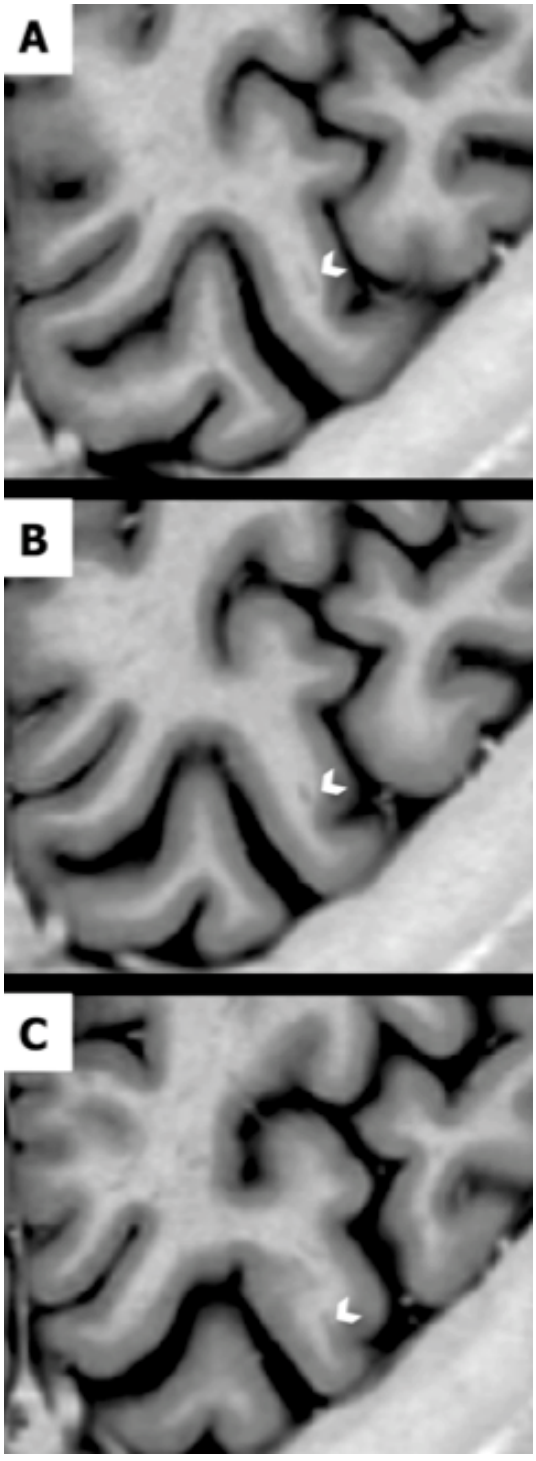
Lesion distribution was tested for normality using a Kolmogorov Smirnov test. As the distribution of lesions was not found to be normal, nonparametric tests were used for further analysis. Wilcoxon signed-rank tests were used to compare PSIR and DIR lesion counts in the MS cohort, and Spearman's rank correlation coefficients were used to determine their association with clinical and cognitive variables. Differences in lesion counts between MS clinical subgroups were investigated with a Mann-Whitney test. SPSS (version 20) was used to perform the statistical analysis, and $p<0.05$ was considered significant. Intra-class correlation coefficient (ICC) was calculated for both intra-rater and inter-rater PSIR lesion counts.

Figure 2.6 Virchow-Robin spaces



Arrows indicate the VR spaces as seen in a healthy control and in a patient with MS

Figure 2.7 Blood vessels – a common artefact



Sequential slices (note white arrow) showing how the linear hypointensity travels forward with very successive slice, indicating it's a blood vessel and not a lesion. The effect of partial volume, due to CSF is a commonly known artefact and is seen especially in the regions of the vertex.

2.4 Results

Demographic and clinical details are provided in Table 2.1 Relapsing remitting MS patients did not differ significantly in age from the controls, but both, the secondary and primary progressive MS patients, were significantly older. Patients with secondary progressive MS had a longer duration of illness than both, relapsing remitting and primary progressive MS (both $p<0.001$). Those with relapsing remitting MS had lower Expanded Disability Status Scale scores than either progressive groups (both primary and secondary progressive, $p<0.001$).The number of different types of lesions, across the different subtypes and controls are detailed in Table 2.4. The infratentorial lesions were counted separately and are recorded in Table 2.5. Control data is recorded in Table 2.6 and 2.7.

The higher-resolution PSIR identified about three times more IC lesions than the lower-resolution DIR in the whole MS cohort ($p<0.001$; Table 2.4). PSIR also identified more LC lesions than DIR ($p<0.001$); this increase was also approximately threefold when JC and LC lesions from PSIR were counted together. It was not possible to separate JC and LC lesions using DIR. PSIR IC lesion count correlated with DIR IC counts ($r_S=0.604$, $p<0.001$), and PSIR LC with DIR LC ($r_S=0.651$, $p<0.001$). In the cerebellum, using DIR, JC lesions could not be separated from the LC lesions. In contrast to the above findings (higher number of CGM lesions detected using PSIR than DIR) a higher number of lesions involving cerebellar cortex are detected using DIR. (DIR CGM> PSIR CGM; $p<0.05$) SP has the highest number of lesions across all lesion subtypes. SP has more lesions than PP (DIR $p=0.15$; PSIR $p=0.05$) and RR (DIR $p=0.02$; PSIR $p=0.02$)(See Table 2.5).

On PSIR, >=1 IC lesion(s) were seen in 60/60 MS patients and 1/30 controls, and >=1 LC lesion(s) in 60/60 patients and 6/30 controls. On DIR, >=1 IC lesion(s) were seen in 50/60 patients and 0/30 controls, and >=1 LC lesion(s) in 60/60 patients and 5/ 30 controls. Curvilinear cortical lesions that followed the contour of gyral and sulcal folds were seen in 36% patients using DIR, and 85% patients using PSIR . Such lesions were not seen in the healthy control group. (See Table 2.6 and Table 2.7)

On PSIR, relapsing remitting and secondary progressive MS patients had similar IC lesion counts, but primary progressive MS patients had significantly lower counts in comparison with both, relapsing remitting ($p=0.023$) and secondary progressive ($p=0.029$) patients. Secondary progressive MS patients also had significantly higher LC lesion counts than primary progressive ($p=0.023$) and relapsing remitting ($p1=0.008$) patients (See Table 2.4) On DIR, relapsing remitting and secondary progressive MS patients had similar IC lesion counts; primary progressive MS patients had significantly lower IC lesion counts than relapsing remitting patients ($p=0.015$). Secondary progressive MS patients also had significantly higher LC lesion counts than relapsing remitting ($p=0.003$) patients.

All MS patients had white matter lesions on their T2 and PSIR scans, with a similar overall mean WM lesion count (See Table 2.4) and there was a close correlation between the two measures ($r_s=0.965$, $p<0.001$). T2 lesion count also correlated with DIR LC ($r_s1=0.558$, $p<0.001$) and PSIR IC and LC

(r_s=0.331, p<0.05 and r_s=0.0647, p<0.001) lesion counts. There was no correlation of CGM lesion counts with disease duration.

Correlations between clinical measures and CGM lesions counts were limited to the relapse-onset (i.e., combined relapsing remitting and secondary progressive) MS cohort (See Table 2.8). Due to the number of p values reported in this analysis, caution must be taken in assessing significance due to the danger of Type 1 error. A bonferroni correction for the number of tests presented in Table 2.8, suggests that the threshold of significance of 0.003 should be required to maintain an overall Type 1 error of 5%. Using this most consistent results are noted when considering the relapse onset group as a whole; the most consistent correlations were between the DIR IC lesion counts and 9-Hole Peg Test (r_s=0.436, p=0.003) and PSIR LC counts with the Symbol Digit Modality Test (r_s=-0.440, p=0.003) (See Table 2.8). None of the six controls with CGM lesion(s) on PSIR had cognitive abnormalities.

The ICC for inter-rater variability for total CGM lesion counts (IC and LC) was 0.955 (p<0.001), and for intra-rater variability it was 0.995 (p<0.001). For IC and LC lesions separately, the ICC for inter-rater variability was 0.681 (p<0.05) for IC and 0.955 (p<0.001) for LC counts, respectively. Intra-rater ICC was 0.990 and 0.984 for IC and LC lesions (both p<0.001) (See Table 2.9a and Table 2.9b).There was a significantly higher grey matter minus white matter contrast (C_{gm-wm}) on PSIR than DIR images (mean=1.48, SD=0.71 versus mean=0.05, SD= 0.03; p<0.05 paired sample t-test).

Table 2.4 CGM and WM lesion counts across study participants

MS Sub-type	DIR		PSIR				PD/T2
	IC	LC	IC	LC	JC	WM	WM
PP (n=15)	3.7 ±4.6	6.1 ±4.8	12.3 ±8.8	11.6 ±12.7	6.1 ±5.5	50.4 ±31.7	48.3 ±27.6
SP (n=15)	6.5 ±5.3	12.2 ±9.6	21.3 ±10.0	20.8 ±13.6	11.1 ±11.2	80.5 ±40.7	72.9 ±33
RR (n=30)	6.6 ±3.9	5.5 ±7.6	19.5 ±9.2	10.5 ±11.4	5.8 ±6.8	63.4 ±57.2	61.2 ±51.2
All (n=60)	5.9 ±4.5	7.3 ±8.0	18.1 ±9.8	13.4 ±12.9	7.1 ±8.1	64.5 ±48.5	60.9 ±42.5

IC –Intracortical , LC –leucocortical, JC –Juxtacortical, WM –white matter , PP – Primary progressive, SP – secondary progressive, RR –relapsing remitting MS. All numbers below represent mean ± standard deviation.

Table 2.5 Cerebellar CGM lesion counts on DIR and PSIR

	DIR			**PSIR**			
	IC	LC	Total	IC	LC	JC	Total
PP	0.3±1.0	1.8±3.2	2.1±3.7	0.3±0.4	1±1.2	0.1±0.4	1.4 ± 1.7
SP	1.6±1.6	2.7±2.2	4.3±3.5	0.9±1.1	2.3±1.8	0.1±0.3	3.2±2.5
RR	0.9± 1.3	1.5± 2.1	2.3±3.2	0.3±0.5	1.2±1.5	0±0	1.5±1.9
Total	0.9±1.3	1.8±2.5	2.8±3.4	0.5±0.7	1.4±11.6	0.1±0.2	1.9±2.1

IC –Intracortical , LC –leucocortical, JC –Juxtacortical, WM –white matter , PP – Primary progressive, SP – secondary progressive, RR –relapsing remitting MS. All numbers below represent mean ± standard deviation

Table 2.6 Control Data

	DIR		PSIR	
	≥ 1 IC	≥ 1 LC	≥ 1 IC	≥ 1 LC
Patients n=60	50	60	60	60
Controls n=30	0	5	1	6

(Above figures represent the number of people with >= 1 IC or LC lesion)

Table 2.7Frequency of curvilinear shaped lesions

	Patients	Controls
DIR	36%	0 %
PSIR	85%	0 %

.

Table 2.8 Correlations between lesion loads and clinical measures.

			RR				SP				RR and SP				PP			
			IC	**LC**	**JC**	**WM**	**IC**	**LC**	**JC**	**WM**	**IC**	**LC**	**JC**	**WM**	**IC**	**LC**	**JC**	**WM**
EDSS	**DIR**	r^2 **p**	0.213, 0.259	-0.173, 0.362,			-0.041, 0.881	**-0.515, 0.049**			0.039, 0.802	0.171, 0.261			-0.354, 0.195	-0.261, 0.347		
	PSIR	r^2 **p**	0.013, 0.947	0.142, 0.454	0.051, 0.789	0.181, 0.338	-0.114, 0.685	-0.451, 0.091	-0.291, 0.293	-0.511, 0.051	0.033, 0.829	0.300, 0.045	0.218, 0.150	0.212, 0.161	-0.378, 0.165	-0.205, 0.464	-0.075, 0.791	0.170, 0.544
MSFC	**DIR**	r^2 **p**	0.282, 0.131	-0.097, 0.611			0.285, 0.302	-0.039, 0.889			0.246, 0.104	0.229, 0.131			-0.066, 0.816	-0.364, 0.182		
	PSIR	r^2 **p**	-0.281, 0.132	0.094, 0.621	0.252, 0.179	0.007, 0.969	0.088, 0.755	-0.014, 0.960	-0.346, 0.206	-0.309, 0.262	-0.068, 0.659	0.265, 0.079	0.225, 0.138	0.026, 0.868	-0.266, 0.337	-0.041, 0.884	-0.295, 0.286	0.179, 0.524
25TWT	**DIR**	r^2 **p**	-0.097, 0.611	-0.239, 0.203			0.137, 0.626	-0.062, 0.826			-0.013, 0.931	0.199, 0.190			-0.217, 0.438	-0.447, 0.095		
	PSIR	r^2 **p**	-0.286, 0.126	-0.150, 0.427	-0.142, 0.456	-0.035, 0.854	-0.031, 0.913	0.037, 0.897	-0.201, 0.473	-0.217, 0.438	-0.124, 0.417	0.095, 0.536	0.028, 0.854	0.035, 0.820	-0.334, 0.224	-0.148, 0.598	-0.182, 0.517	0.369, 0.175
9HPT	**DIR**	r^2 **p**	0.393, 0.032	-0.027, 0.889			0.546, 0.035	-0.056, 0.844			**0.436, 0.003**	0.075, 0.625			-0.101, 0.721	0.038, 0.893		
	PSIR	r^2 **p**	0.111, 0.557	0.403, 0.027	0.183, 0.333	0.067, 0.724	0.458, 0.086	-0.032, 0.909	-0.307, 0.266	-0.379, 0.163	0.239, 0.114	0.211, 0.163	0.045, 0.770	-0.064, 0.676	-0.238, 0.394	0.291, 0.293	-0.113, 0.689	0.386, 0.155
PASAT	**DIR**	r^2 **p**	0.107, 0.575	-0.115, 0.547			-0.162, 0.563	0.058, 0.830			0.009, 0.953	-0.057, 0.710			0.099, 0.726	-0.224, 0.421		
	PSIR	r^2 **p**	-0.371, 0.044	-0.068, 0.723	0.132, 0.487	-0.064, 0.737	-0.269, 0.333	-0.022, 0.939	-0.116, 0.680	-0.149, 0.596	-0.362, 0.015	-0.059, 0.700	0.039, 0.801	-0.106, 0.489	-0.050, 0.861	-0.166, 0.555	-0.520, 0.047	-0412, 0.127
SDMT	**DIR**	r^2 **p**	-0.274, 0.142	-0.244, 0.194			-0.227, 0.455	0.069, 0.822			-0.228, 0.142	-0.278, 0.071			-0.042, 0.882	-0.071, 0.802		
	PSIR	r^2 **p**	-0.332, 0.073	-0.449, 0.013	-0.095, 0.616	-0.276, 0.140	-0.412, 0.162	-0.047, 0.878	-0.095, 0.759	-0.022, 0.943	-0.365, 0.016	**-0.440, 0.003**	-0.221, 0.155	-0.312, 0.042	-0.052, 0.853	-0.332, 0.226	-0.365, 0.181	-0.516, 0.049

Table 2.9 Calculation of Inter-user (a) and Intra-user (b) variability

SNo.	User 1			User 2			User 3			(A) Inter-User Variability
	IC	**LC**	**IC+LC**	**IC**	**LC**	**IC+LC**	**IC**	**LC**	**IC+LC**	
1	33	113	146	6	85	91	37	61	98	
2	22	67	89	2	61	63	22	18	40	
3	15	47	62	26	24	50	64	10	74	
4	14	74	88	9	46	55	8	62	70	
5	1	11	12	0	4	4	5	1	6	
6	1	12	13	1	3	4	12	5	17	
7	3	28	31	2	11	13	8	7	15	
8	20	175	195	5	103	108	28	94	112	
9	0	4	4	1	1	2	2	2	4	
10	21	51	72	4	33	37	19	20	39	
SNo.	**Trial 1**			**Trial 2**			**Trial 3**			**(B) Intra User Variability**
	IC	**LC**	**IC+LC**	**IC**	**LC**	**IC+LC**	**IC**	**LC**	**IC+LC**	
1	41	71	112	37	61	98	37	78	115	
2	36	12	48	22	18	40	24	24	48	
3	62	19	81	64	10	74	59	21	80	
4	14	54	68	8	62	70	12	44	66	
5	5	0	5	5	1	6	4	1	5	
6	18	4	22	12	5	17	11	9	20	
7	14	8	22	8	7	15	11	14	25	
8	24	75	99	28	94	112	26	78	104	
9	2	1	3	2	2	4	2	2	4	
10	15	30	45	19	20	39	16	35	51	

Above table indicates raw data for CGM lesions across 10 participants. Table 2.9a – data for three separate users; 2.9 b – data for same user (myself), recorded three times.

2.5 Discussion

The aim of this work was to compare PSIR with DIR as a sequence to detect CGM lesions and to study correlations of lesion number with clinical and cognitive measures of disability. These data suggest that PSIR does indeed improve the detections of CGM lesions by about 3x. This was true for both intracortical and leucocortical lesions. In the cerebellum however, the number of CGM lesions detected was greater using DIR than PSIR. As expected by studies of natural history, (Ebers & Daumer 2008; Scalfari et al. 2010; Weinshenker et al. 1989) patients with secondary progressive disease had a significantly longer duration than either primary progressive / relapsing remitting and the patients with progressive disease were older than the controls and RRMS patients(Table 2.1).

As mentioned, secondary progressive patients had the highest lesion load and this was expected. Interestingly while the number of intracortical lesions in secondary progressive and relapsing remitting patients was similar, the number of leucocortical lesions in secondary progressive patients was almost double that of RR. This is interesting as it suggests either that 1) leucocortical lesions continue to accrue with time or that 2) intracortical lesions keep accruing, but also evolve into leucocortical lesions. This would need to be verified using longitudinal studies but this could help understand whether the primary pathology is in the white or grey matter.

All patients had at least one intracortical lesion but this was seen only in one control with PSIR. This contrasting finding suggests that intracortical lesions may be specific for MS patients. DIR also identified these intracortical

lesions but given that this was true only of 50/60 patients as compared to 60/60 for PSIR, it suggests that PSIR is probably more sensitive in identifying them. None of the controls had an intracortical lesion on DIR, and this was in accordance with other reports. (Roosendaal et al. 2009; Ciccarelli 2012)

The occurrence of the curvilinear shape also seems to be characteristic for patients with MS. Earlier work by Calabrese et al. has shown the frequency of these lesions to be about 25%(Calabrese et al. 2007); my findings were similar at 36% on DIR. The identification of a curvilinear morphology on PSIR, together with the identification of an intracortical lesion in a patient, may help in the differential diagnosis of MS when suspected, but not confirmed.

Given that at least 11 of the patients had a disease duration of < 5 years, this suggests that intracortical lesions are seen very early in the disease and could perhaps help to categorise and prognosticate patients with clinically isolated syndromes. A recent study of biopsy specimens has also suggested a high frequency of demyelinating CGM lesions in patients subsequently confirmed to have MS. (Lucchinetti et al. 2011)

Correlations between white matter count on PSIR and PDT2 suggest that PSIR might effectively replace the latter for the purposes of estimating white matter lesion load. Thus, in addition to being a high resolution sequence at a clinically acceptable time, it is effectively giving information about both grey matter and white matter. This is in effect replacing DIR (for grey matter) and FLAIR (for white matter).

Histopathological studies have shown subpial lesions as being the most dominant type. On PSIR, while pure intracortical lesions were identified,

these were limited to the deeper layers of the cortex / seemed to involve the entire thickness of the cortex. Pure subpial lesions were not seen. This could be possibly due to (i) very small size and low contrast of subpial lesions or (ii) Perhaps the subpial lesions are being counted as intracortical lesions. Future imaging work that allows us to segment the cortex and see the exact layers of the cortex that are affected in a lesion, may allow us to separate the subpial lesions from the intracortical lesions.

On looking at correlations between the CGM lesion counts and the clinical measures, lesion counts on DIR correlated with the 9HPT, which is a test of motor function. LPM (mapping (Calabrese et al. 2010a) work done using DIR noted that the CGM lesions were largely localised to the region of the frontotemporal lobes; particularly in the motor regions. This may explain the correlations of CGM lesion counts with tasks of motor function such as the 9HPT. PSIR detected CGM lesion counts on the other hand, correlated with SDMT, which is a test of cognitive dysfunction. It is possible that PSIR is sensitive for CGM lesion detection in other regions and that different regions are preferentially identified by these two techniques. A better understanding of these findings may emerge when regional lesion counts are compared for PSIR and DIR sequences. To understand this further, similar work would need to be done on PSIR. In addition, the counts of lesions in different lobes may have better correlations with specific cognitive tests and this needs to be explored in future.

In patients with primary progressive MS, CGM lesion counts were smaller than in those with relapse onset MS. This is similar to findings on T2

white matter lesion counts. Only 15 primary progressive patients were part of this cohort and a larger cohort should be studied to understand the implication of this observation.

Why did I choose to compare PSIR with 2D DIR? There are other sequences including 3D MPRAGE and 3D DIR which give useful information. Because DIR has been recently accepted as a modality that helps increase GM lesion detection, I used a sequence with different resolution and contrast mechanisms for this comparison. However as has been highlighted in other work, different scans maybe more sensitive at picking up different aspects of the pathology of the disease and perhaps the combined use of different sequences will help us understand and identify pathology better.

As shown in Figure 2.1, an increased resolution of six times (from a voxel size of 3 mm^3 on DIR to 0.5mm^3 on PSIR) is achieved. While other techniques like using multislab scanning with DIR, 3D DIR etc. may help in the future; resolution/unit time achieved with PSIR remains a strong qualitative advantage of PSIR. As scan times increase, problems such as motion artefacts do also tend to increase, especially in a cohort of patients with progressive disease. It also needs to be remembered that PSIR (in 11 minutes) provides information about grey matter (DIR 4 minutes) and white matter (FLAIR 7 minutes). Thus in a similar amount of scan time, it will be possible to use just one sequence to give us information about both grey and white matter.

In an attempt to bridge the gap between detection of grey matter lesions seen histopathologically and on imaging, PSIR shows potential to aid this measure. On comparison with the current gold standard, DIR, there is a

significant increase in the quantity and quality of detection of lesions using PSIR.

While earlier work with DIR has shown correlations between lesion volume an cognitive measures, this needs to be reproduced with PSIR to establish if cognitive impairments can in fact be predicted by the number and distribution of CGM lesions.

As mentioned, this cohort included some patients who were on disease modifying drugs. Do these drugs affect the number / evolution of lesions? Can the distribution of lesions be used to ascertain the point of starting / stopping treatment? These answers require long-term follow up of these patients, with serial scans and analysis models that account for the specific disease modifying therapy.

By studying patients longitudinally, PSIR has the potential to study the evolution of lesions. In an interesting observation, the contrasting finding between patients and controls, suggest that the detection of an intracortical lesion and a curvilinear morphology, may have a role to aid in the differential diagnosis of patients with MS, when a diagnosis is suspected but not confirmed. Should histopathological correlation of PSIR findings be achieved, this would be a possible strong argument to include detection of GM lesions as one of the criteria considered in the diagnosis of this neuroinflammatory condition.

Using post mortem verification, the sensitivity of DIR to detect CGM lesions has been reported to be 18%, as compared to almost ~9% with FLAIR. (Seewann et al. 2012) Given that PSIR improved detection by almost three fold, the sensitivity of PSIR can be predicted to be around 50%.

Retrospective comparisons had improved the sensitivity from 18% to 37% in the study done by Seewan et. al. Based on the above analysis, the combined analysis of DIR and PSIR, could likely further improve the sensitivity to detect CGM lesions.

The greater number of cerebellar CGM lesions being detected on DIR, suggest differences in the sensitivity of both sequences, when comparing supra-tentorial vs. infratentorial regions (Gilmore et al. 2009). There are various reasons why this maybe possible. From an anatomical point of view, the structure of the cerebellar cortex is less compact in comparison to the cerebral cortex. Flow artefacts due to the vicinity to blood vessels and motion artefacts are other factors that possibly contribute to the low CGM detection on PSIR. Conventional MRI scans are also noted to demonstrate differences in sensitivity for different regions of the brain. For the study of lesions in deep grey matter and the posterior fossa, PD/T2 is preferred in comparison to FLAIR. The difference in T1 contrast for different tissues may contribute to this difference, and also to the above reported difference in PSIR/DIR for the study of CGM lesions. The different degree of demyelination noted between the cerebellar and the cerebral cortex are also likely to affect the use of different sequences. Lastly, most brain protocols are optimised using the brain (cerebral cortex) as the centre, not the cerebellum; and this together with above features might contribute to the difference reported above.

These results suggest that with the use of PSIR, the detection of CGM lesions is improved by about 3x as compared to DIR. In addition, the improved resolution allows more confident classification of lesions, allowing the separation of JC lesions separate from the LC lesions. Like with double

inversion recovery, post mortem verification of CGM lesions will truly help understand the sensitivity and specificity for detection of CGM lesions using PSIR.

Refining the guidelines for lesion identification using PSIR will help minimize the number of lesions missed. However in order to improve the sensitivity and specificity of these guidelines, similar analysis should be reproduced across centres and a consensus reached over regions more /less likely to be ‘lesional’. A retrospective PSIR to DIR analysis would not provide any additional information and is limited by the extent of lesions seen on PSIR.

3 Comparison of classification of lesions from DIR to PSIR

3.1 Abstract

Objective

Accurate identification and localization of CGM lesions in MS is important when determining their clinical relevance. Double inversion recovery (DIR) scans have been widely used to detect MS CGM lesions. Phase sensitive inversion recovery (PSIR) scans have a higher signal to noise, and can therefore be obtained at a higher resolution within clinically acceptable times. This enables detection of more CGM lesions depicting a clearer cortical and juxtacortical anatomy. In this study, I systematically investigated if the use of high resolution PSIR scans changes the classification of CGM lesions, when compared with standard resolution DIR scans.

Methods:

60 patients (30 RR(Relapsing remitting) and 15 each with PP(Primary progressive) and SP(Secondary progressive) MS) were scanned on a 3T Philips Achieva MRI scanner. Images acquired included DIR (1x1x3mm resolution) and PSIR (0.5x0.5x2mm). CGM lesions were detected and classified on DIR as intracortical (IC) or leucocortical (LC). I then examined these lesions on corresponding slices of the high resolution PSIR scans and categorized them as IC, LC, Juxtacortical white matter (JC-WM, abutting but not entering cortex) and other white matter (WM, not juxtacortical).Classifications using both scans were noted.

Results:

282 IC and 483 LC were identified on DIR. Of the IC lesions, 61% were confirmed as IC on PSIR, 35.5% were reclassified as LC and 3.5% as JC-WM or other WM only. Of the LC DIR lesions, 43.9% were confirmed at LC on PSIR, 16.1% were reclassified as IC and 40% as JC-WM or other WM only. Overall, 50% (381/765) of CGM lesions seen on DIR were reclassified, and 26.5% (203/765) affected WM only.

Conclusions:

When compared with higher resolution PSIR, a significant proportion of lesions classified as involving CGM on DIR appear to either contain more white matter than expected or to not involve CGM at all.

3.2 Introduction

Double inversion recovery (DIR) (Seewann et al. 2012; Calabrese, et al. 2010a; Geurts et al. 2005b) has been most extensively used to study CGM lesions in vivo, and it has been shown that accrual of CGM lesions relates to disability in established MS (Calabrese et al. 2010a; Geurts et al. 2005b) and their presence may also improve the specificity of current MS MRI diagnostic criteria (Chapter 2) (Filippi et al. 2010b).

MS CGM lesions have been histopathologically sub-classified based on (1) location i.e. relative to GM/CSF and GM/WM boundary and (2) morphology. Based on their location they have been sub-classified as abutting the cerebrospinal fluid (CSF) but not extending through the whole cortical ribbon, traversing the full thickness of the cortex from the CSF to WM interfaces, or confined to the centre of the cortex (not touching either the CSF or WM boundaries)(Chapter 2) (Filippi et al 2010b; Geurts et al. 2011; Bø et al 2003b).

Using DIR it is difficult to reliably identify subtypes of GM lesions (Chapter 2) In particular, juxtacortical WM lesions (JC-WM) - one of the subtypes of WM lesions that count towards MS MRI diagnostic criteria (Nelson et al. 2011; Geurts, Pouwels, et al. 2005b; Polman et al. 2011) - have the potential to be confused with mixed GM-WM lesions using DIR. In comparison, high resolution PSIR scans detect about three times as many CGM lesions, as shown in the previous chapter. In addition, they considerably improve the anatomical depiction of the cortical ribbon and adjacent WM, and can separate juxtacortical WM (JC-WM) lesions from mixed GM-WM lesions (Chapter 2) (Lazeron et al. 2000). These lesion subtypes have been studied

in context of their diagnostic role (Calabrese, et al. 2011a; Filippi et al 2010b) and also in relation to their association with cognitive impairment (Chapter 2) (Papadopoulou et al. 2013; Calabrese et al. 2011a) but to be reliable, such analyses depend on accurate lesion subtype identification.

CGM lesions have also been divided into subtypes based on morphology, on DIR (Nelson et al 2007a; Calabrese et al. 2010a) and PSIR (Seewann et al. 2012; Lucchinetti et al. 2011). More recent work has suggested that curvilinear lesions may help differentiate age-associated GM lesions from those due to neuroinflammation (Chapter 2) (Calabrese, Battaglini, et al. 2010a; Bø et al. 2003b; Geurts et al. 2005b).

In Chapter 2, the results are indicative that PSIR helps improve the detection of CGM lesions as compared to DIR. In order to study the effects of these lesions on clinical and cognitive morbidity, and further ascertain the contribution of both grey and white matter, the accurate identification and localization of CGM lesions in MS is important. As suggested by observation in Chapter 2, cortical and juxtacortical anatomy is more clearly visible using PSIR. The separation of JC from LC lesions is significant as it not only helps study the effects of JC lesions, but also prevents the misclassification of a lesion on the basis of its grey / white matter involvement. In this chapter, I outline a systematic investigation of the change of classification of cortical and juxtacortical lesions on using the high resolution PSIR scans, in comparison to DIR.

I hypothesized that the higher resolution of PSIR will improve the accuracy of classifying CGM lesions in clinically acceptable scanning times. A

better understanding of the extent of grey matter involvement and possibly the morphology of lesions, will improve the specificity of observations made in the context of MS and reduce the noise while interpreting correlations between CGM lesion and parameters of cognitive and clinical parameters.

Different groups have investigated the roles of DIR (Calabrese, Battaglini, et al. 2010a; Geurts, Pouwels, et al. 2005b; Geurts et al. 2011), PSIR (Filippi et al 2010b) and a combination of DIR and PSIR in improving lesion detection (Chapter 2). (Geurts et al. 2011; Nelson et al. 2007a; Bø et al. 2003b; Nelson et al. 2011). A comparison of lesion classification across these scans has not been studied before.

3.3 Methods

3.3.1 Participants and Image acquisition

Scans collected from the same cohort of 60 patients {30 relapsing-remitting (RR) MS, 15 each with primary (PP) and secondary progressive (SP) MS} recruited in the earlier part of this project (Chapter 2) were now studied to investigate the change in classification of lesions across both these scans. Image acquisition and was performed as described in Chapter 2.

3.3.2 Image Analysis

CGM lesions were identified and classified on both DIR and PSIR scans as described in Chapter 2. (Geurts et al. 2011). With an aim to study the change in classification from DIR to PSIR, I then examined these CGM lesions seen on DIR, on corresponding slices of the high resolution PSIR Scans; subtype classification in relation to the grey–white boundary was noted. All lesions were marked under supervision of senior raters (including

an experienced neuroradiologist).

The CGM lesions detected on DIR (IC or LC) were classified on PSIR scans in to one of 4 categories: IC, LC, JC-WM lesions (WM lesions abutting but not entering the cortex), or other WM lesions (non-JC). It can be recalled that JC lesions could not be separated from LC lesions on DIR, but this was possible on PSIR. Based on their morphology, CGM lesions were sub-classified by shape as curvilinear (lesions that follow the contour of sulcal and gyral folds), oval or wedge shaped (Chapter 2) (Calabrese, Battaglini, et al. 2010a).Classification of DIR visible CGM lesions was noted using DIR and then at the corresponding location seen on PSIR. I noted changes in classification from either IC / LC on DIR to IC/LC/JC or WM on PSIR.

3.4 Results

765 CGM lesions were marked on DIR, 282 of these were IC and 483 LC. Of the 282 IC lesions, 172 (61%) were confirmed as IC on PSIR, and 110(39%) were reclassified (100 LC (35.5%), 8 JC-WM (2.8%) 2 Non-JC WM (0.7%)). Of the 483 LC DIR lesions, 212 (43.9%) were confirmed as LC on PSIR, and 271 (56.1%) were reclassified (78 IC (16.1%), 155 JC-WM, 38 WM (i.e., 40% involved WM only) (See Table 3.1 and Figures 3.1 to 3.4)

Morphologically, on DIR, 665 of CGM lesions were initially classified as oval, 59 curvilinear and 41 wedge shaped. Using PSIR 148/765 (19.3%) were reclassified. The most striking difference was the higher number of lesions classified as curvilinear on PSIR than on DIR. (See Table 3.2 and Figure 3.2)

Table 3.1: Change in lesion subtype, from DIR to PSIR

Change in classification based on type of lesions					
		As subsequently seen on PSIR			
		IC	LC	JC	WM
On DIR	IC (N=282)	172(61)	100(35.5)	8(2.8)	2(0.7)
	LC (N=483)	78 (16.1)	212 (43.9)	155(32.1)	38 (7.9)

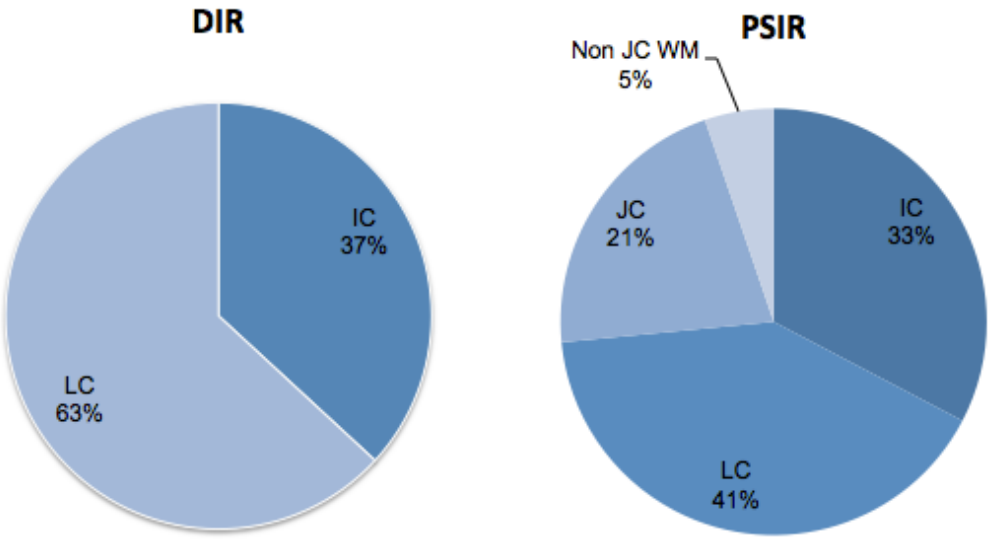
(number (percentage)) Each row corresponds to a lesion type seen on DIR, and each column how the same lesion was classified on PSIR. E.g. of the IC lesions so classified on DIR, 60% remained so on PSIR

Table 3.2 Change in lesion morphology, from DIR to PSIR

Change in classification based on Morphology of lesions				
		As subsequently seen on PSIR		
		Oval	Wedge	Curvilinear
On DIR	Oval (N=665)	523 (78.6)	26 (3.9)	116 (17.4)
	Wedge (N=41)	0	30 (73.2)	11(26.8)
	Curvilinear (N=59)	5 (8.5)	0	54 (91.5)

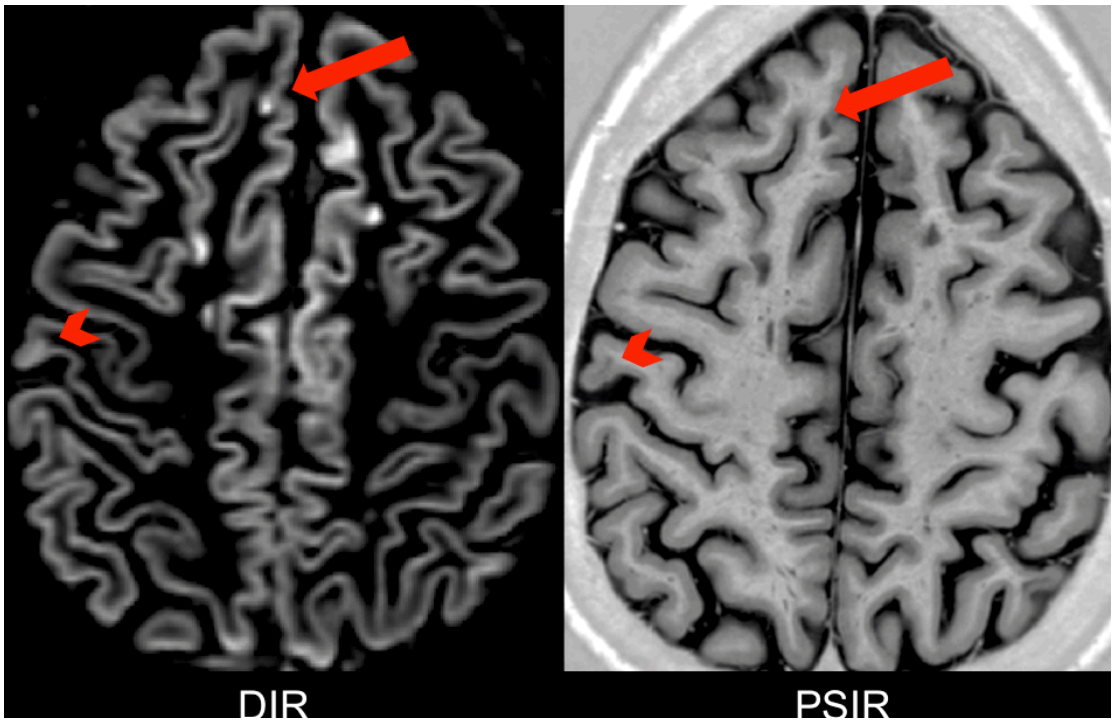
(number (percentage)) of lesion numbers based on morphology of lesions, as seen on DIR and PSIR

Figure 3.1 Conversion of lesion classification from DIR to PSIR



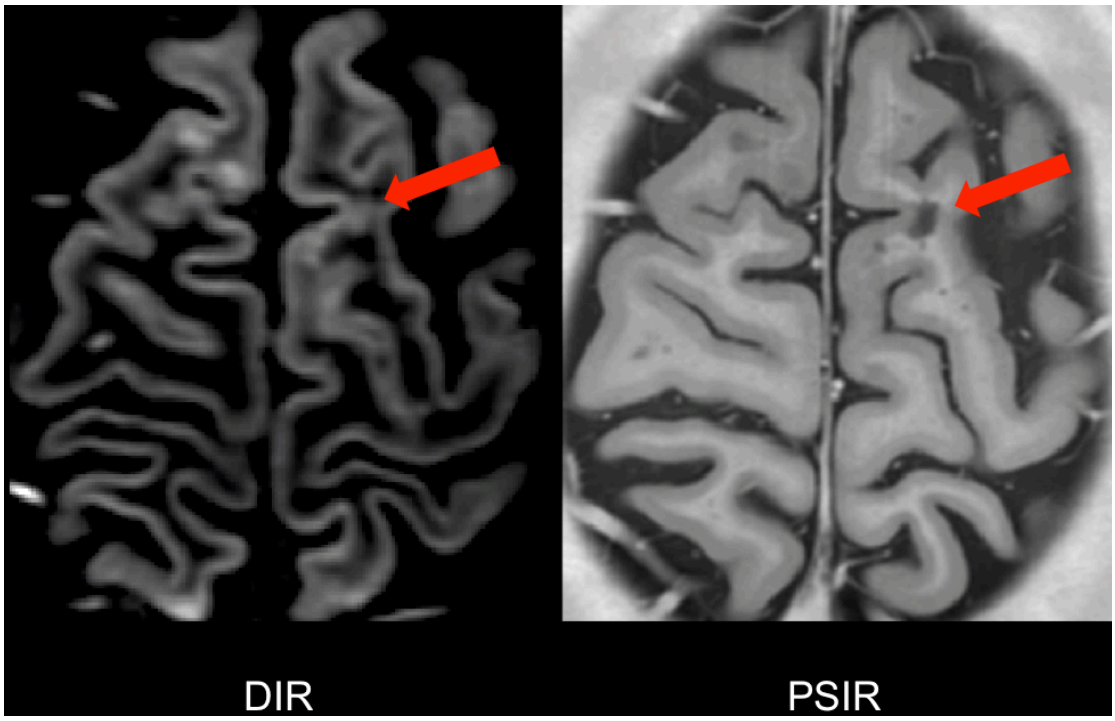
The pie chart on the left indicates % of IC and LC lesions using DIR; on the right is the figure with revised % of lesions using PSIR

Figure 3.2Corresponding images on DIR and PSIR



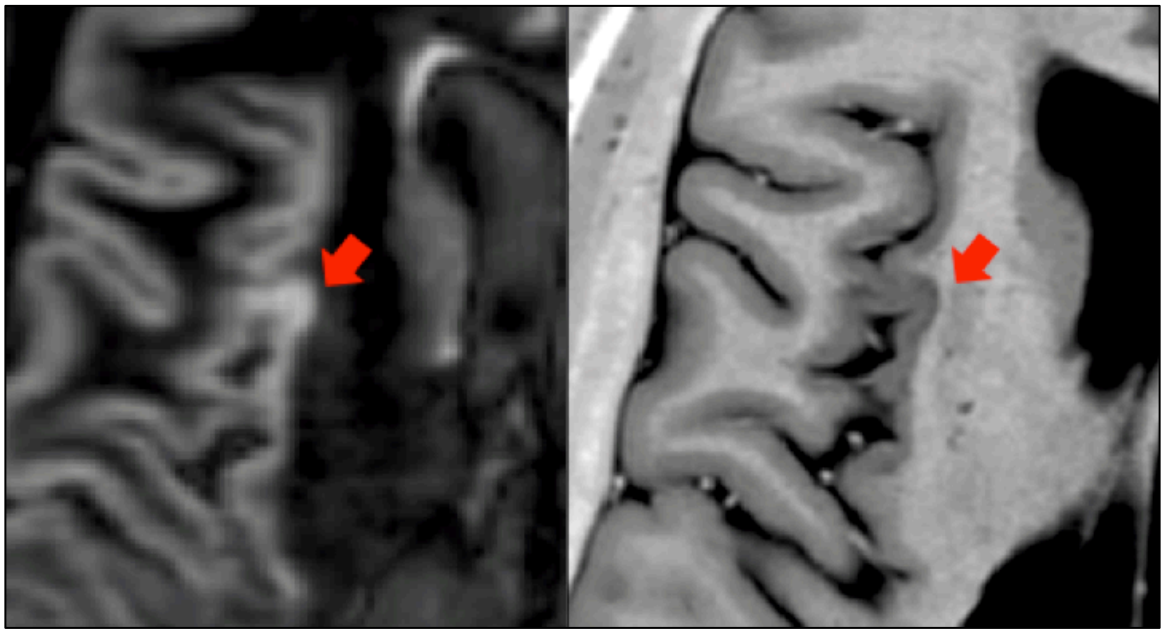
LC lesions on DIR (arrow and chevron on left) appears to be a JC white matter lesion on PSIR (arrow and chevron on right); The lesion shown by the arrow appears oval on DIR abut wedge shaped on PSIR

Figure 3.3Corresponding images on DIR and PSIR



LC lesion on DIR (left)appears to be a limited to white matter on PSIR (right)

Figure 3.4:Corresponding images on DIR and PSIR



LC lesion on DIR appears to be an IC on PSIR

3.5 Discussion

In this study I found that MS lesions reported to involve the CGM on DIR were often reclassified in a different anatomical location when viewed on a higher resolution PSIR sequence. Specifically, 39% (110/282) lesions thought to only involve CGM on DIR (i.e. IC lesions) where noted to extend into WM on PSIR, and of the mixed GM-WM lesions on DIR (i.e. LC) about56 % (271/483) were reclassified on PSIR, with about 40% (193/483) classified as being purely in the WM.

A recent combined histopathological and MRI study reported that 90% of CGM lesions seen on a high resolution 3D DIR scan (using a scan resolution of 1.1 by 1.1 by 1.3 mm, which is higher than those commonly used in vivo) were histopathologically confirmed (Filippi et al. 2010b; Seewann et al. 2012). However, the present study suggests that at the resolution that has been more often employed in clinical DIR studies published to date (1x1x3mm3) a larger proportion of DIR identified CGM lesions may actually be false positives.

In Chapter 2, the results indicate that when CGM lesions were marked independently on DIR and high resolution PSIR, about three times more CGM lesions were detected on PSIR . (Papadopoulou et al. 2013; Calabrese et al. 2011a). However, the high frequency in the present study with which DIR CGM lesions were reclassified as WM lesions suggests that the increase in true CGM lesion detection on PSIR may be even higher, perhaps about 6 times that of DIR.

Not surprisingly, DIR-classified LC lesions were more likely than IC

lesions to be reclassified as being entirely in the WM (40% and 3.5% respectively). JC-WM lesions seen on conventional T2-weighted and FLAIR sequences are included in the current McDonald 2010 diagnostic criteria (Calabrese, et al. 2010a; Polman et al. 2011) but CGM lesions seen on DIR have been suggested as more predictive of conversion from a clinically isolated syndrome to MS (Chapter 2) (Filippi et al. 2010b). Intracortical lesions have been suggested as having a role in diagnostic criteria (Bø et al. 2003b; Filippi et al. 2010b) and accurate sub-classification into lesion subtypes, will have important implications when using GM lesions to improve MS MRI diagnostic criteria.

A question arises whether the diagnostic value of lesions in this region is coming from true CGM lesions, or JC-WM lesions, or both. Prospective studies following CIS patients who have had high resolution PSIR scans at presentation could address this question. Higher resolution DIR scans also warrant investigation as they could potentially also improve accuracy of lesion classification compared with the standard resolution DIR scans that were investigated in this study.

Cognitive impairment, is seen in about 40 – 65% patients with MS. Previous work using standard resolution DIR has demonstrated correlations with measures of cognitive and clinical disability supporting a role for lesions in or near the cortex in determining clinical outcomes(Geurts, Pouwels, et al. 2005b; Nelson et al. 2011; Geurts et al. 2011), while study using FLAIR scans reported an association of cognitive impairment with JC-WM lesions (Chapter 2) (Lazeron et al. 2000) The observed limited accuracy of DIR again raises questions about the relative clinical and cognitive effects of true CGM and JC-

WM lesions. Accurate sub-type classification is important in establishing the association between CGM lesion number and volume with cognitive impairment(Nelson et al 2007a; Calabrese et al. 2011a; Nelson et al. 2011). A higher CGM lesion number has been reported in those MS patients who are cognitively impaired and CGM lesion volume has been found to be an independent predictor. Accurate classification and quantification of lesional grey matter is key in studying whether this impairment is driven by pathology in the grey/ white matter.

Curvilinear lesions have been reported to be seen in 36% of people with MS (using DIR) and in 85% patients using PSIR. In contrast, no similar morphology was seen in healthy controls (Chapter 2). Given the frequency of this lesion morphology, I was keen to understand the difference in the above percentages across both scans. Of the CGM lesions seen on DIR, 27% of the wedge shaped and 17% of the oval shaped lesions were reclassified as curvilinear on PSIR. Thus on reclassification a more accurate morphological categorization is also facilitated using PSIR. However, it is important to study larger cohorts and also compare with other neurological disease groups in order to ascertain the specificity and implications of these findings.

In conclusion, these data suggest that lesions classified as involving CGM on standard resolution DIR images are reclassified as WM-only lesions when viewed on a higher resolution PSIR sequence. While DIR has improved CGM lesion detection, PSIR, which further improves detection, is in addition more representative of the actual grey matter lesional load.

This finding cautions against using DIR in isolation, for the classification of CGM lesions. In future the multimodal approach, using

multiple scans like DIR and PSIR, may help with lesion detection (Geurts et al. 2011; Nelson et al. 2007a) (Chapter 2) but PSIR is a more robust sequence for accurate classification of lesion subtype and morphology. The findings of this work warrant attention while studying the role of CGM lesions in MS diagnostic criteria and in investigating how elements of CGM pathology affect cognitive or neurological function. While post mortem verification of CGM lesions using DIR (Calabrese et al. 2010a; Seewann et al. 2012; Lucchinetti et al. 2011) has recently been studied, similar verification of PSIR visible CGM lesions will allow an improved understanding of these results.

4 Cortical and Juxtacortical lesions in multiple sclerosis: their distribution on MRI and impact on cognition

4.1 Abstract

Background

The spatial distribution of CGM lesions in multiple sclerosis (MS) is predominantly fronto-temporal in histopathological studies. CGM lesions have been associated with cognitive impairment and progressive disability in MS. PSIR (Phase sensitive inversion recovery) MRI has improved the detection of CGM lesions and has the potential to improve the characterization of regional distributions of CGM lesions in vivo. CGM and juxtacortical (JC) white matter (WM) lesions have both been linked with cognitive dysfunction in multiple sclerosis (MS). Little is known about their relative associations with cognition, and if such associations vary across cortical lobes and MS phenotypes.

Aim

To determine the lobar distribution of CGM and JC lesions and their associations with cognitive deficits in people with different MS subtypes.

Methods

Using a Philips 3T Achieva system, PSIR scans (0.5x0.5x2 mm^3) were acquired for 30 people with relapsing remitting (RR), 15 primary (PP) and 15

secondary progressive (SP) MS. On the PSIR scans intracortical (IC) or leucocortical (LC) CGM lesions and JC WM lesions were identified. Lesion volumes were estimated for frontal, temporal, parietal, occipital lobes. A frontal cognitive z-score was derived from Hayling and Stroop test z-scores; a temporal cognitive z-score was computed using immediate and delayed story recall, and immediate and delayed complex figure recall; an attention z-score was calculated using Symbol digit modality test (SDMT) and Paced auditory serial addition test (PASAT). Differences between phenotypes and associations with cognitive measures were examined using multiple regression to adjust for potential confounders. Global WM lesion volume, age, gender, disease duration, pre-morbid IQ and intracranial volume were included as covariates.

Results

Mean (standard deviation) CGM lesion volume (mm3) was 434.3 (528.5) in the frontal, 272.9 (280.1) in the temporal, 142.0 (184.0) in the parietal and 17.2 (54.2) in the occipital lobes. Performance in the frontal cognitive tests was not found to be associated with frontal lesion volumes (IC , LC or JC). Larger temporal JC lesion volume was associated with a lower temporal cognitive z-score ($p<0.001$). A larger frontal IC lesion volume ($p=0.019$) and a higher parietal LC lesion volume ($p=0.073$) were independently associated with a lower attention z-score. No differences in these associations were noted between clinical subgroups.

Conclusion:

This imaging study identified a fronto-temporal dominance and occipital paucity of CGM lesions. Different CGM and JC lesion subtypes are associated with cognitive function, and their relationship is influenced by the lobar location of lesions and the cognitive function being assessed.

4.2 Introduction

Histopathological studies have demonstrated a fronto-temporal predominance of CGM lesions, albeit in samples from people with mostly long-standing progressive disease (Nichtweiss et al. 2012; Brownell & Hughes 1962; Assareh et al. 2010; Lumsden 1970). Such lobar predilections may, in part, explain the pattern of cognitive deficits seen in people with MS but it is not clear whether or not the relative lobar distribution of GM lesions is the same throughout the clinical course of MS. Further, lesions involving GM may also extend into WM, and it is not clear if the previous associations between GM lesions and cognitive function that have been observed (Calabrese et al. 2009) (Giorgio & De Stefano 2010) are mostly driven by the GM or WM component of such lesions (Hulst et al. 2013; Mueller et al. 2010; Papadopoulou et al. 2013).

Technological limitations have made it difficult to reliably detect GM lesions in vivo, although a substantial improvement was achieved (when compared with conventional magnetic resonance imaging (MRI) techniques) with the introduction of double inversion recovery (DIR). However DIR yields images which have a low signal to noise compared with conventional MRI methods, and it is very difficult to determine reliably whether or not a lesion is confined to the cortex, extends into the WM, or abuts but does not enter the cortex (a juxtacortical (JC) WM lesion). Building on this, I have demonstrated the use of a high resolution phase sensitive inversion recovery (PSIR) (Chapter 2) sequence, originally used to confirm lesions seen on DIR (Nelson et al 2007a) . PSIR increased GM lesion detection by two to three fold

compared to DIR (Chapter 2) and enabled CGM lesion subtypes (intracortical (IC) lesions confined to CGM and leucocortical (LC) lesions involving both CGM and JC-WM) and JC WM lesions to all be separated more robustly (Chapter 3)

Radiologically detected WM lesion load correlates only modestly (Calabrese et al. 2009; Kacar et al. 2011; Barkhof 2002a; Bakshi et al. 2008; Barkhof et al. 2009a; Gonzalez et al. 1994) with the extent of disability seen in patients with MS leading to a clinical – radiological paradox (Giorgio & De Stefano 2010; Barkhof 2002a). It has been hypothesized that CGM lesions might be responsible for the clinical presentation of patients, and may be the missing piece of the puzzle. (Hulst et al. 2013; Calabrese, Rinaldi, Poretto, et al. 2011b; Mueller et al. 2010; Chard & Miller 2009; Papadopoulou et al. 2013). However with conventional imaging, sub-classification of lesions and detection of CGM lesions has been limited. By improving contrast between the grey and white matter boundary, and lesional and non-lesional tissue, studies using DIR and PSIR have been able to study the clinical impact of cortical and juxtacortical lesions.

Cognitive impairment is often seen in people with MS, (Nelson, Poonawalla, Hou, Huang, Wolinsky & Narayana 2007a; Bobholz & Rao 2003; Rao et al. 1991) affecting executive tasks, memory, attention and processing efficiency. People with progressive forms of MS have more cognitive dysfunction than patients with relapsing remitting (RR) disease. The impact of topographic distribution of WM lesions on pattern of cognitive deficit has been studied (Tiemann et al. 2009; Sperling et al. 2001; Gonzalez et al. 1994). Both cortical and subcortical lesions have also been implicated as a possible

pathological substrates (Kutzelnigg & Lassmann 2006; Rovaris & Filippi 2000; Calabrese, Rinaldi, Grossi, et al. 2011a) (Roosendaal et al. 2009) of cognitive dysfunction and in recent years. Modest associations between cognitive impairment and global CGM lesion volume (Calabrese et al. 2009), intracortical (Nelson et al. 2011) leucocortical (Nielsen et al. 2013) and juxtacortical (Lazeron et al. 2000) lesions have been reported. Other measures such as cortical atrophy (Giorgio & De Stefano 2010) and MTR (Summers et al. 2008) have also been investigated with regards to their correlation with cognitive impairment. The distribution of GM lesions has been associated with specific patterns of cognitive impairment. (Polman et al. 2011; Tiemann et al. 2009; Nielsen et al. 2013). However, there has been little investigation to elucidate the relative contribution of the distribution of CGM lesion subtypes (IC, LC, JC) (Chapter 2). Using PSIR, accurate detection of IC and LC lesions (Chapter 3) now allows us to explore if these lesions subtypes are responsible for cognitive impairment.

In order to study the relationship of CGM and JC-WM lesions with cognitive impairment, it is important to use reliable and accurate measures of these parameters. The distribution of lesions can be studied based on their location (i) in the anatomically defined lobes (frontal, temporal, parietal, insular and occipital) and (ii) through the thickness of the cortex. Based on their locations within the thickness of the cortex, CGM lesions are pathologically classified into four distinct types, Type I -IV. Type I lesions are situated at the GM/WM junction, furthest away from the CSF. On the other hand, the subpial lesions (Type III) are adjacent to the CSF and furthest away from the white matter. Type II lesions are surrounded by GM in their entirety and Type IV

lesions extend all the way from the subpial surface to the GM/WM boundary (Bø & Bø 2009). Radiologically, using PSIR, it is possible to classify lesions in and adjacent to the cortex as LC (equivalent to Type I), IC (Types II- IV) and JC (Chapter 2).

In this work I assessed: (i) the distribution of IC and LC CGM lesions, and JC WM lesions, across lobes; (ii) the associations of lesion type and location with cognitive dysfunction; (iii) whether or not distributions and associations (i and ii) differed between MS subtypes.

4.3 Methods

4.3.1 Participants

Sixty people (30 Relapsing remitting (RR), 15 primary progressive (PP) and 15 (SP) MS) with a clinically definite diagnosis of MS,(Polman et al. 2011) no other known neurological condition, and aged between 18 and 65 years, were enrolled as part of an earlier study assessing the use of PSIR for CGM lesion detection. (Chapter 2; Table 2.1). All participants provided a written informed consent for this study which was approved by the local institutional ethics committee.

4.3.2 Image acquisition and analysis

A Philips 3T Achieva (Philips Healthcare, Best, The Netherlands) system was used to acquire images for this study, using a 32-channel head coil. The protocol included FLAIR, PSIR and the volumetric T1-weighted gradient echo (3DT1)sequence. Details of acquisition parameters are given in (See Chapter 2, Table 2.2)

4.3.3 Marking and classifying GM lesions

Guidelines for defining CGM lesions using PSIR were as previously described in Chapter 2. CGM were subdivided into IC or LC; and WM lesions touching but not entering the cortex were classified as juxtacortical (JC) lesions. All other white matter lesions (non-JC) were also marked separately. Marking of all scans was carried out blinded to clinical features. Lesion marking and contouring was done using the software JIM v 6.0. Both number and volume of lesions was determined.

4.3.4 Estimating lobar lesion counts

Frontal, temporal, parietal, insular and occipital lobe masks in MNI152 space from the Wake Forest University Pick-Atlas (Maldjian et al. 2003) were registered to each participants' PSIR via the volumetric T1-weighted image. All registrations were carried out using NiftyReg (http://sourceforge.net/projects/niftyreg) (Modat et al. 2010). The steps for this included the following :

Step 1 : Creating a template brain

To account for brain atrophy (in particular enlarged ventricles) seen in MS, a cohort specific brain template was built from the T1w images from patients in the cohort. The 3D T1 scans had all been lesion filled. This was generated by repeatedly creating an average of the T1w images and registering each image to the average. This was done initially using linear registration and then refined by non-linear registration. The process was repeated 9 times to obtain a clear template.

Step 2 : Registrations (See Figures 4.1, 4.2 and 4.3)

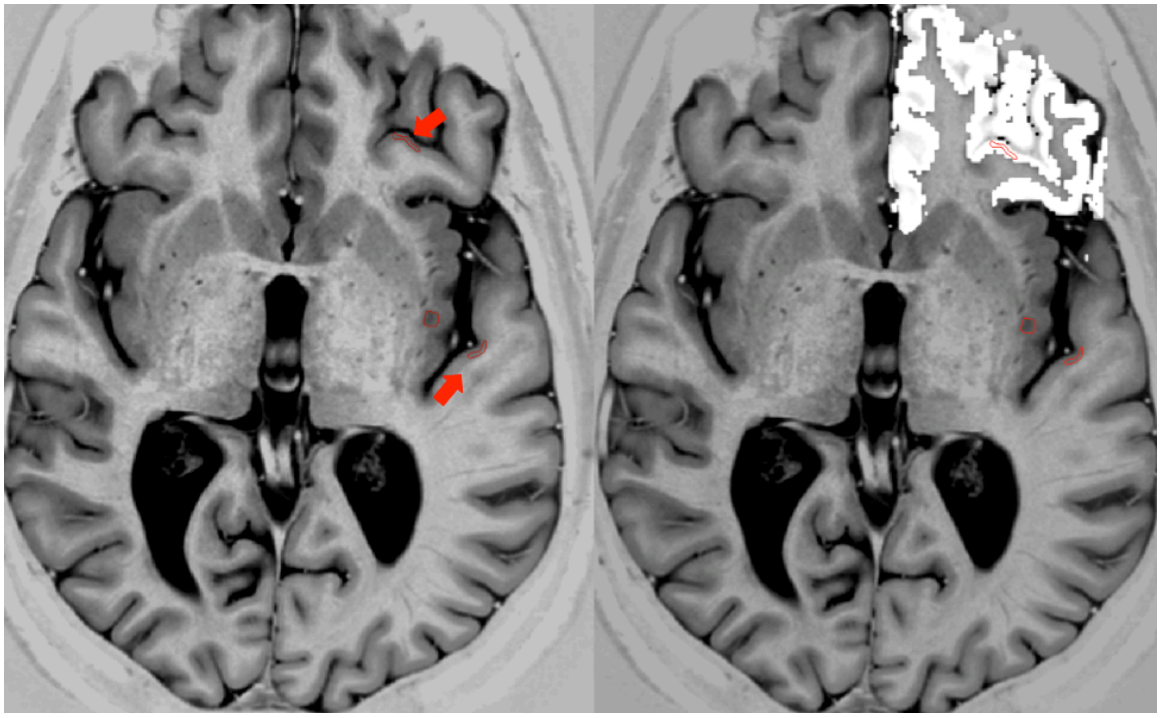
i. The MNI152 1 mm T1w template brain was non-linearly registered to the cohort specific template brain.
ii. The cohort specific template brain was then non-linearly registered to the individual participants' T1w scan.
iii. Each individual participant's T1w scan was rigidly-aligned with their PSIR scan.
iv. The transformation fields from steps i, ii and iii were concatenated and used to transform the lobar masks from MNI space into native PSIR space.

To enable the relative proportion of lesion to normal-appearing (NA)GM to be calculated in each lobe, i.e. to determine if any differences in lobar lesion loads were simply due to the differences in the size of the lobes, lobe specific GM masks were generated :

v. The T1w images were segmented using unified segmentation in SPM8 into GM, WM and CSF tissue classes. These were binarised using a maximum likelihood algorithm (Muhlert et al. 2013). The binarised grey matter mask was then transformed into native PSIR space using the parameters from step iii.
vi. PSIR space lobar masks were intersected with GM masks from step v, to create lobar GM masks.
vii. For each participant a total intracranial volume measurement was calculated by first binarising the tissue volumes using a maximum likelihood algorithm, then summing their volumes (i.e. grey matter + white matter + CSF). This was done to normalise the grey matter volume measurements.

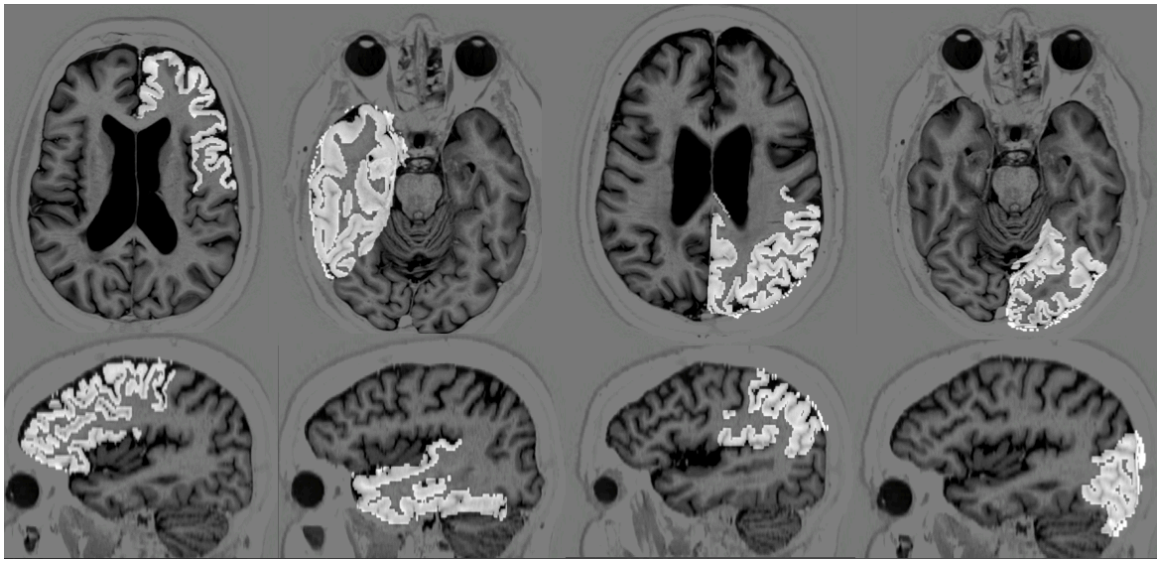
Using in house software, lobar number and volume of each lesion type (IC/LC/JC) was determined. Lobar volumes were calculated and divided by each participants' total intracranial volume. (calculated by summing the volumes from each of the binarised tissue classes from step v). Lesion density measured by the lesion volume as a proportion of the lobar volume was also calculated.

Figure 4.1 Overlaying lobar masks in PSIR space



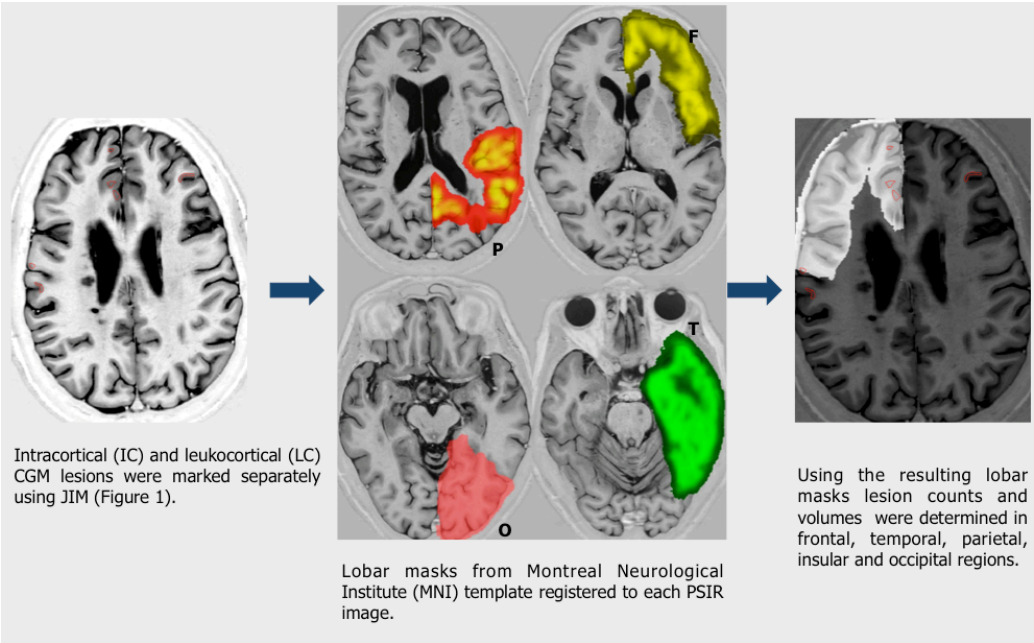
PSIR image showing marked CGM lesions (1A) and overlayed left frontal lobe mask(1B)

Figure 4.2 Overlaying lobar masks in PSIR space



Axial (top panel) and sagittal (bottom panel) PSIR images with lobar masks (registered from MNI-152 brain template to PSIR); frontal, temporal, parietal and occipital lobes (left to right)

Figure 4.3 Estimating lobar lesion counts



Above figure combines the steps in figures 4.1 and 4.2 ; Once the marked region of interest file was superimposed on the lobar masks, the lesions for each lobe were then quantified.

4.3.5 Clinical and cognitive assessment

A brief medical and a more detailed neurological history was recorded for all participants. A clinical examination, similar to a normal neurology outpatient clinic review was performed by an experienced clinician. Their Expanded Disability Status Scale (Kurtzke 1983) and MSFC (MS Functional Composite) (Cutter et al. 1999; Fischer et al. 1999) scores (comprising the 9HPT(9 hole peg test), 25TWT (timed walk test) and PASAT (Paced auditory serial addition test)) were also determined. The individual components were analysed as Z scores: for PASAT by standardizing the raw score to using the group mean and standard deviation, for the other two components by standardizing the reciprocal of the mean times, after substituting a zero for subjects not completing the tasks

Detailed cognitive assessment was performed by an experienced neuropsychologist (NM), for each participant. The cognitive functions examined were grouped into three major domains: (i) executive function (ii) memory and (iii) information processing speed / attention.

Executive function was tested using the Hayling sentence completion and Stroop tests. The Hayling sentence completion test and Stroop colour-word (Van der Elst et al. 2006) interference test were used to assess vulnerability to interference. To assess memory, immediate and delayed figure recognition and immediate and delayed story recall tasks from the Adult Memory and Information Processing Battery (Coughlan AK, Hollows SE, 1985; The Adult Memory and Information Processing Battery (AMIPB), Leeds, St James' University Hospital) were used. For the information processing speed/attention composite measure, PASAT and symbol digit modalities test

(SDMT) (Smith 1982) were used.

All cognitive measures were converted into z scores by subtracting from sample means and dividing by sample standard deviations. For the purposes of analysis, the three domains, executive, memory and information processing/attention were analysed together 1) “executive”, comprising the Hayling and Stroop measures 2) “memory”, comprising four measures: the immediate and delayed figure recognition and story recall tasks; 3) “attention” comprising two measures, PASAT and SDMT. A composite z score was calculate for each of the three by averaging across the individual z scores.

4.3.6 Statistical Methods

Lesion volumes tend to be positively skewed and thus render unreliable some analyses which assume normality. One approach is to normalise by log transformation; however, this is not advisable when, as in these data, there are many zero volumes which have no valid logarithm. For linear regression, however, the normality assumption applies only to residuals, not to individual regression variables, and often a skewed response variable may still give rise to normal residuals. The approach taken here therefore is, where linear regression is employed, to use the untransformed lesion volume, but where there was evidence of residual non-normality, to confirm or replace results with a bootstrapped regression which makes no normality assumption.

Spearman correlation was used to investigate if, for a given lobe, patients with higher lobar volume also had higher lesion volume in that lobe. While this would be a correlation between patients, a different question is whether within patients their larger lobes (e.g. frontal and temporal) contain

larger lesion volumes than their smaller lobes (e.g. insula and occipital): this involves a type of ‘paired’ test. since lobes are not independent within patients, and was performed by testing for within-patient variation in lesion density across lobes using a multivariate regression with five lobe density response variables, jointly testing equality of the five mean densities.

Lesion density variables and proportions of lesions in a lobe or location were compared between patient groups using multiple linear regression on group indicators with disease duration and gender covariates; when age was substituted for duration results were not materially altered. To compare absolute lesion volumes aggregated over lobes between phenotypes, intracranial volume was entered in addition to duration and gender. To compare lobe-specific absolute lesion volumes between phenotypes, the relevant lobar volume was entered in addition to duration, gender and intracranial volume.

MSFC component scores rather than the composite were analysed for two reasons: firstly, because of the separate information conveyed by the components, particularly, in this context, the PASAT. Secondly, because the composite standardly requires the walk times to be used, which are almost always skewed, and this skew persists in the standard composite. By analysing the reciprocal of the walk times (walk speed) the measure is normalized (Kapoor et al. 2010).

It was hypothesised that the “attention” and “executive” measures may be associated with lobar (frontal, temporal) lesion variables. Due of the relatively large number of individual cognitive measures, in order to address

hypotheses with a relatively small number of statistical tests, three multivariate multiple regressions were used, for each domain simultaneously regressing their individual cognitive measures on lesion predictors. This allows joint testing of the null hypothesis that none of the individual domain measures is associated with the lesion predictors. By reporting individual associations only where the corresponding joint test is significant for the domain, there is less danger of spurious significant results. Additionally, the domain-averaged single z scores were examined in a single multiple regression, though this loses the measure-specific information. For these cognitive measure regressions, potential confounders entered as covariates were age, gender, pre-morbid IQ, intracranial volume and lobar volume.

Regression residuals were examined for normality and where there was evidence of non-normality results were checked using a non-parametric bootstrap with 1000 replicates. Analyses were performed in Stata 13.1 (Stata Corporation, College Station, Texas, USA).

4.4 Results

The demographics of patients in this study have been detailed in Chapter 2 (Table 2.1). The WM lesion and intracranial volume measures for the cohort are reported in Table 4.1

4.4.1 Distribution of lesions

Distribution of Lesions in the whole cohort

All results below are reporting lesion volume. Lesion density and lesion number results were reflective of similar results and are reported as supplementary data. (See 4.6) Lesion volumes for IC, LC and JC lesions were

found to be the highest in the frontal and lowest in the occipital lobes (See Table 4.2, Figure 4.4).The mean percentage of GM lesions in each lobe for the whole group of patients are frontal 43.2%, temporal 28.4%, parietal 15.7%, insular 9.4% and occipital 3.2%.

Distribution of lesions between MS subtypes

The total IC lesion volume (in mm^3) across all lobes, was significantly higher in SP than PP (adjusted difference in means 233.478, 95% confidence interval (CI) 3.620, 463.336; P=0.047), but not than RR (P=0.198); LC lesion volumes were substantially and significantly greater in SP than both PP and RR (adjusted difference in means respectively 525.769, 95% CI 95.836, 955.702; P=0.017, and 621.094 95% CI 233.115, 1009.073; P=0.002). SP had double the JC lesion volume than PP and RR. There was also a trend for patients with SPMS to have a higher WM lesion count and volume than those with RRMS or PPMS, but this did not reach statistical significance. The CGM lesion volume (IC and LC volumes combined) was significantly higher in SP than PP or RR (adjusted difference in means respectively 776.333, 95% CI 207.622, 1345.044; P=0.008 and 756.827, 95% CI 243.612, 1270.042; P=0.005).

Table 4.1 Measure of white matter lesion load and other covariates

Type	PSIR WM lesion number	PSIR WM lesion volume (mm^3)	Age (years)	M:F	Disease duration (years)	ICV (mm^3)	GMF
RR (n30)	63.9 (57)	6881.9 (10949)	42.5 (9.6)	5:10	11.5 (10.5)	1460.9 (156.2)	0.47 (0.01)
PP (n15)	50.1 (31.8)	6486.8 (8046.3)	51.9 (9.1)	7:8	12.1 (8.0)	1475.1 (149.5)	0.47 (0.02)
SP (n15)	79.5 (38.9)	7482.6 (5424.2)	50.9 (7.6)	10:20	23.3 (7.3)	1431 (150.7)	0.47 (0.01)

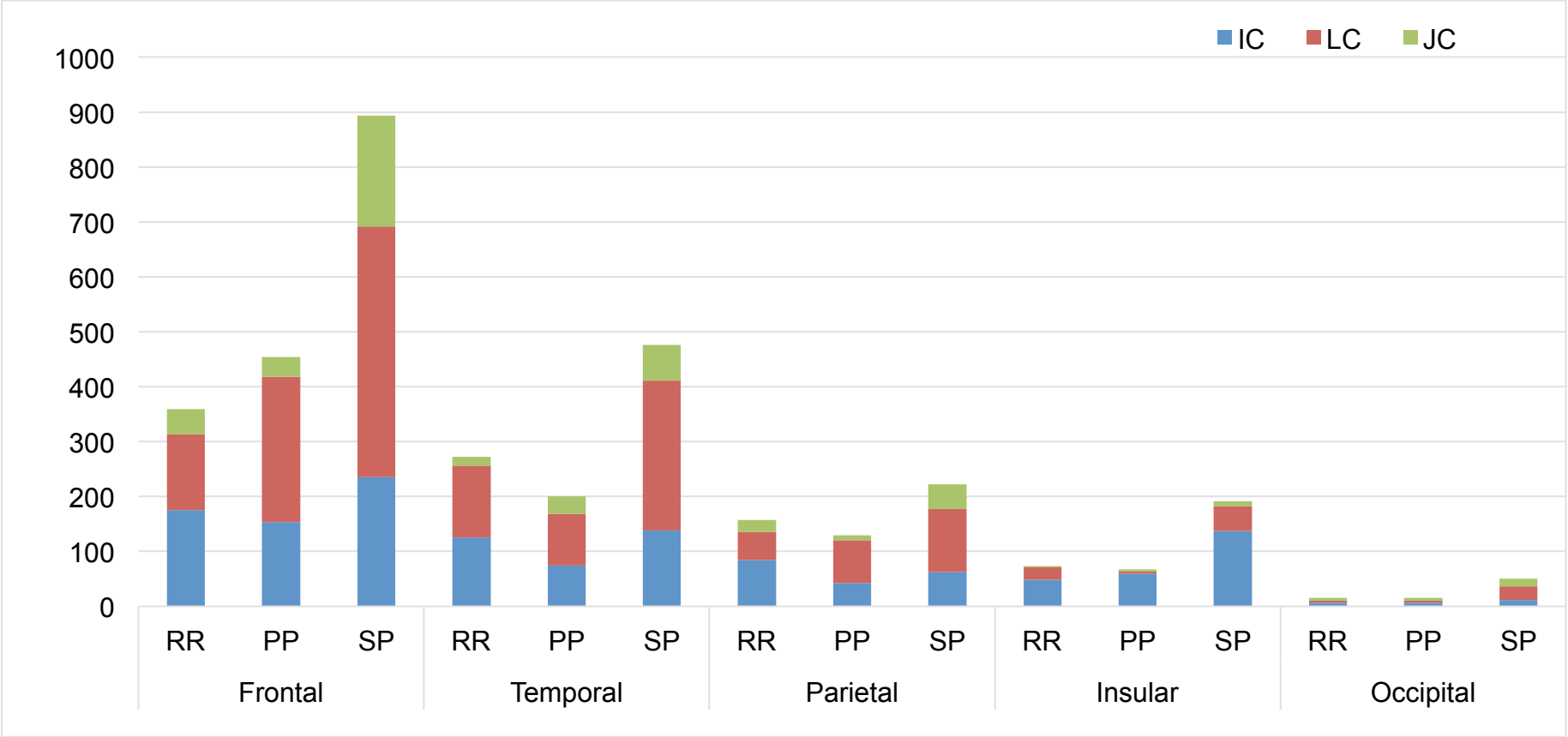
Above data reported as mean (SD); PSIR –Phase sensitive inversion recovery, GMF – grey matter fractions, ICV – Intracranial volume , M- Males, F- Females

Table 4.2 Distribution of CGM lesions (Volumes)

	Frontal			Temporal			Parietal			Occipital			Insular		
	IC	LC	JC	IC	LC	JC	IC	LC	JC	IC	LC	JC	IC	LC	JC
RR (n30)	175 (114.2)	138.7 (241)	45.9 (73.8)	125.9 (101.8)	130.6 (169.4)	15.6 (21.7)	84 (64.9)	51.4 (75.5)	22 (33.8)	6.8 (17.7)	4.2 (10.9)	4.4 (10)	48.7 (99)	22.7 (59.5)	2 (8
PP (n15)	153.3 (143.7)	264.5 (404.5)	36.4 (47.4)	74 (66.6)	94.3 (93.1)	32.4 (52)	42.4 (55.2)	77.8 (121.9)	9.5 (13.1)	6.1 (13.5)	4.2 (12.1)	4.9 (9.7)	59.2 (110.3)	4.6 (10.1)	3.1 (11.
SP (n15)	235.2 (234.8)	457.1 (466.9)	201.1 (261.1)	138.5 (78.7)	272.4 (251.4)	64.5 (118.8)	62.4 (75)	114.6 (169)	45.6 (94.8)	11 (29.6)	25.9 (64.2)	13.8 (29.2)	137.1 (159.7)	45 (67)	8.1 (15.
All (n60)	184.6 (159.2)	249.7 (369.3)	82.3 (155.5)	116.1 (90.8)	157 (189.5)	32 (68)	68.2 (66.6)	73.8 (117.4)	24.8 (53.9)	7.7 (20.2)	9.6 (34.1)	6.9 (17)	73.4 (123.1)	23.8 (55.1)	4.1 (11.

Lesion volumes reported as Mean (SD) in mm^3

Figure 4.4 Distribution of lesions (By MS Phenotype)



Above graphs shows the distribution of lesions (IC, LC and JC) across the five lobes (frontal, temporal, parietal, insular and occipital) in the three phenotypes of MS patients (RR, PP and SP). All above data is based on lobar lesion volume

Distribution of IC, LC and JC lesions between lobes

Within patients, lobar volumes decreased in the order: frontal, temporal, parietal, insula, occipital; however, the lesion volume contained in the lobes did not decrease proportionately, as reflected in a significant variation in lesion density across the lobes (P<0.0001): frontal and temporal lobes have similar density, 0.2%, while parietal (0.1%) and occipital (0.04%) have lower densities and insula the highest (0.7%). However, there was no evidence to suggest that, for a given lobe, patients with larger lobes also had higher lesion volume in that lobe: for example, patients with larger frontal lobes did not contain significantly higher frontal lesion volume than patients with smaller frontal lobes.

Comparing lesion locations across the five lobes studied, SPMS had significantly higher lesion volume than both PP and RR for: frontal JC (adjusted difference in means vs. PP, 120.608, 95% CI 8.826, 232.389 P=0.035; vs. RR 104.099, 95% CI 2.857, 205.3412 P=0.044); occipital LC lesion volume (adjusted mean difference in means vs. PP 27.116, 95% CI 0.264, 53.967 P=0.048; vs. RR 27.432, 95% CI 3.170, 51.693 P=0.027). SPMS had a higher frontal LC than RRMS (adjusted difference in means vs. RR 321.050, 95% CI 76.240, 565.860 P=0.011) but not PPMS (195.931, 95% CI 74.36353, 466.2257 P=0.152). Comparison between subtypes for other lobes and locations were not significantly different.

4.4.2 Association between lesion volumes and measures of cognition

Frontal Cognitive measures (See Table 4.3; Table 4.4)

There was no evidence that that either of the two cognitive measures was associated with WM lesion volume (P=0.186 for joint test). Nor was there evidence they were associated with the frontal IC volume (P=0.121 for the joint test). Nor was there evidence that these cognitive measures were associated frontal LC or JC lesion volumes or with lesion volumes from the other lobes. There was also no evidence that the lesion density was more strongly associated than the absolute volume.

Temporal Cognitive measures (See Table 4.3 Table 4.5)

The temporal z score was significantly ($p<0.001$) associated with temporal JC volume, adjusting for potential confounders. The joint test of association between the four temporal cognitive measures and temporal JC lesion volume was highly significant ($p<0.0001$) with significant evidence of negative association in all four. The adjusted standardized regression coefficients for story immediate, story delayed, figure immediate and figure delayed were respectively -0.373 (95% CI -0.563, -.183 P<0.001), -0.403 (95% CI -.584, 0.223 P<0.001) -0.469 95% CI -0.657, -0.282 P<0.001) and -0.489 (95% CI -0.510, -0.089 P=0.005).

There was no evidence that temporal JC lesion density was a better predictor than absolute volume. There was no evidence of association with the temporal IC or LC volumes, independent of the JC volume; nor was there evidence of association, independent of temporal JC volume with volume

measures in other lobes. There was no evidence that any of the four temporal cognitive measures was associated with WM lesion volume (p=0.584 on the joint test).

Attention scores (See Table 4.3 Table 4.6)

Frontal IC volume and Parietal LC volume were independently negatively associated with the attention measures. In a model with both lesion measures as predictors (and adjusting for potential confounders), the joint test of association gave P=0.035 for Frontal IC and P=0.023 for Parietal LC. For Frontal IC the adjusted standardized regression coefficients for PASAT and SDMT respectively were -0.239 (95% CI -0.447, -0.030 P=0.025) and -0.222 (95% CI -0.409, -0.035 P=0.020); and for parietal LC, -0.099 (95% CI -0.323, 0.125 P=0.387) and -.278 (95% CI -0.470, -0.086 P=0.005) respectively. This suggests the parietal LC volume may be more strongly associated with SDMT than PASAT. The attention Z score was also significantly associated with frontal IC volume (P=0.019) and borderline significantly associated with parietal LC (P=0.073), adjusting for potential confounders.

There was no evidence that lesion densities were more strongly predictive than absolute volumes. There was no significant association between WM lesion volume and attention scores (p=0.575 for the joint test)

Table 4.3 Mean (SD) Z Scores for cognitive measures

	z_front	z_temp	z_attention
RR (n30)	0.1 (0.8)	0.3 (0.7)	0.2 (0.8)
PP (n15)	-0.1 (0.7)	-0.3 (0.9)	-0.2 (0.8)
SP (n15)	-0.1 (0.8)	-0.5 (1.1)	-0.2 (1.0)
All (n60)	-0.01 (0.8)	0.0 (0.9)	0.0 (0.9)

Z_front : Z score of cognitive tasks used to assess frontal lobe function, z_temp : Z score cognitive tests used to measure temporal lobe function and z_attention – z score of PASAT and SDMT tasks

Table 4.4 Z Scores for individual tests for Frontal Lobe function

	z_hayling	z_inv_stroop
RR (n30)	0.0 (1.1)	0.3 (1.0)
PP (n15)	0.0 (1.0)	-0.3 (1.0)
SP (n15)	0.1 (0.9)	-0.2 (0.8)
All (n60)	0.0 (1.0)	0.0 (1.0)

Z score for Hayling (z_hayling) and stroop (z_inv_stroop) tasks that were part of assessment of frontal lobe function

Table 4.5 Z Scores for individual tests of Temporal lobe Function

	z_story_imm	z_story_del	z_figimm	z_figdel
RR (n30)	0.2 (0.9)	0.3 (0.8)	0.4 (0.9)	0.4 (0.8)
PP (n15)	-0.1 (1.0)	-0.1 (0.9)	-0.5 (1.0)	-0.4 (1.1)
SP (n15)	-0.4 (1.2)	-0.4 (1.3)	-0.3 (1.0)	-0.4 (1.0)
All (n60)	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)

Z score for immediate (z_story_imm) and delayed (z_story-del) story recall and for immediate (z_figimm) and delayed (z_figdel) figure recognition; these tests comprised assessment of the temporal lobe function

Table 4.6 Z Scores for MSFC and SDMT

	z_inv_walk	z_inv_9HPT	z_pasat	z_sdmts
RR (n30)	0.6 (0.8)	0.2 (0.7)	0.0 (1.0)	0.4 (0.8)
PP (n15)	-0.2 (1.0)	0.1 (1.0)	-0.1 (1.1)	-0.3 (0.8)
SP (n15)	-0.9 (0.7)	-0.5 (1.4)	0.1 (0.9)	-0.5 (1.2)
All (n60)	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)

Z score of 25 foot timed walk (z_inv_walk), 9HPT (z_inv_9HPT), PASAT (z_pasat) and the SDMT (z_sdmt) tests; the former three are part of the MSFC score

4.5 Discussion

The results indicate a fronto-temporal dominance and occipital paucity of CGM lesions. There was no evidence to suggest that, for a given lobe, patients with larger lobes had higher lesion volume in that lobe. Frontal IC volume and Parietal LC volume were negatively associated with the attention measures and deficits in memory function were found to be associated with temporal JC lesion volume. These were independent of whole brain WM lesion load, demonstrating that different CGM and JC lesion subtypes are relevant to cognitive function, but the exact relationship depends on where the lesions are present and which cognitive function is being assessed.

Distribution of lesions

A greater proportion of CGM lesional volume was seen in the frontal (43.2%) and temporal (15.7%) lobes. Occipital lobe contributed the least (3.2%) to the CGM lesional volume. This is in keeping with similar observations made in histopathological (Lumsden 1970; Brownell & Hughes 1962) and neuroimaging studies (sparing of the lateral occipital gyrus (Calabrese, Battaglini, et al. 2010a)).

CGM (IC plus LC) was as expected (Bjartmar et al. 2003; Lassmann et al. 2012), higher in SP than both PP and RR. However, on looking at subsets, the IC lesion load was not significantly different in SP and RR; the difference in CGM lesional volume, is largely driven by differences in LC lesion count. A possible explanation for the differences between SP and RRMS is that there is a shift in new GM lesion formation from being centered on the cortex in RRMS to the cortico-WM junction in SPMS. Alternatively, CGM lesions continue to form in both RR and SPMS, but extend into the

adjacent WM more often in SPMS. Longitudinal studies looking at the evolution of CGM lesions are limited and these would be needed to better understand these findings.

JC lesion loads were also higher in SP than RRMS, contributing to the increase in pathology seen at the GM/WM boundary. Histopatholgical data (Brownell & Hughes 1962) and recent imaging studies (Nielsen et al. 2013) suggest a predominance of lesions at the junction of the GM/WM boundary and SP (vs. RR) patients had significantly higher lesional volume at the boundary. However, it needs to be examined whether these findings also reflect an enlargement of existing lesions to the boundary region (from adjacent cortex or white matter) or represent a true increase of new LC and JC lesions. Longitudinal studies looking at the evolution of CGM lesions are limited and these would be needed to better understand the phenotypical differences in CGM lesion load.

Association between lesion volumes and measures of cognition

The commonly affected cognitive domains in MS include attention / processing speed; learning /memory and executive function. In earlier work with PSIR (Chapter 2) I had noted only modest correlations between global CGM lesion numbers and cognitive performance assessed using the MSFC.

The results of my lobar analysis indicate a poor association between executive function and frontal lobe lesions. It is possible that executive function may be affected by CGM lesions not seen on PSIR (especially subpial lesions) or by other structural and functional changes in normal appearing frontal grey matter. In addition, impaired executive function is reported following damage to a wide network, including the thalamus and

cerebellum, which weren't assessed in this study. Future work can assess the additional predictive value of NAGM changes in MTR, diffusion measures etc., and in damage within broad networks.

The temporal cognitive measures studied were significantly associated with temporal JC volume. The extent and severity of white matter damage (Hulst et al. 2013), presence of juxtacortical lesions (Lazeron et al. 2000) and lesions in the underlying U fibers (Miki et al. 1998) have all been reported to be associated with cognitive impairment in previous studies. It is possible that the temporal lobe functions that were assessed are more network driven and thus affected by WM lesions.

The results suggest that memory function is more sensitive to temporal lobe lesions than executive function is to frontal lobe lesions, which may have implications for the relative plasticity of these functions, i.e., the potential for adaptation by other brain regions when there is pathological involvement of a region that has a key role in mediating a particular cognitive function.

The association of lower attention z-scores with both frontal IC and parietal LC lesion load is consistent with a contribution of more widely distributed CGM lesions to this also common manifestation of cognitive dysfunction in MS.

While the cerebral cortex is recognized as a highly organized seat of control, the relation between a CGM location of lesions and an effect on behaviour / cognition can still not be confidently predicted (Lezak et al. 2012). Interpretation of location – functional correlations is made difficult as the same function can involve different regions of the brain, and in turn each CGM

location may be responsible for many tasks. Thus, it is important to observe caution while investigating the functional localization of cognitive deficits

Limitations

This study has several limitations. Firstly, the MRI sequence used to detect GM lesions in this study, while improving detection when compared with DIR, is still probably missing a substantial proportion of subpial CGM lesions. About 50-65% of CGM demyelination has been reported to be present in the subpial region. This is largely in regions such as the insula, cingulate, frontobasal and temporobasal cortices, and the cerebellum (Bø, Vedeler, Nyland, Trapp & Mørk 2003b; Lassmann et al. 2007; Albert, Antel, Brück & Stadelmann 2007b) In my study however, I was not able to identify any subpial lesions. Radiological visibility of these lesions is limited by their location, size and intrinsic properties (minimal inflammation, lack of a blood brain barrier disruption and a lesser degree of contrast between the lesional /non-lesional cortex). The proximity of the lesions to the CSF also causes susceptibility artefacts. (Schmierer et al. 2010) It is also possible that some of the subpial demyelination (especially that which extends to the WM boundary, as Type IV lesions) has been counted in my dataset as IC. The effect on cognition of CGM lesions may be relatively underestimated, when compared with WM lesions (including JC lesions), that are much more readily identified (Geurts & Barkhof 2008). Secondly, the SP and PPMS groups are smaller than the RRMS group, and this will have reduced sensitivity to subtle phenotypic differences in lesion distributions and associations with cognitive outcomes, although this is unlikely to have significantly influenced the main findings of this work.

In conclusion, my data is in consensus with pathological studies that previously reported fronto-temporal predominance of CGM lesions. This is not simply due to differences in the size of the cortical lobes but represents a true predilection for lesion formation in these regions. Interestingly, when comparing people with RR and SPMS, while CGM lesion loads are higher in SPMS this appears to be mostly due to higher LC rather than IC lesion burden, which may reflect either the enlargement of pre-existing IC or JC into LC lesions, or the more frequent development of new LC lesions as a person with MS moves from RR to SPMS. The specific associations observed in my study between lesions and cognitive measures suggests that the presence, extent and regional location of CGM/JC lesions may all contribute to the patterns of cognitive impairment that occur in MS.

4.6 Supplementary results

Lesion numbers

Lesion numbers for IC, LC and JC lesions were found to be the highest in the frontal and lowest in the occipital lobes (See Table 4.7). The association between lesion numbers and measures of cognition followed the same pattern as noted as noted for lesion volume. However the associations were stronger for volume than for number of lesions.

Lesion Density (lesion volume as a proportion of the lobar volume)

SP had a significantly higher frontal lesion density than both RR and PP (adjusted difference in mean density respectively 0.00214, 95%CI 0.007, 0.003 p=0.002; 0.001, 05% CI 0.0001, 0.003 p=0.034). In the temporal, insular and occipital lobes, the lesional density was significantly greater in SP than PP, but not RR: adjusted differences in mean density vs. PP was 0.001,

95% CI 0.0003, 0.003 p=0.015; 0.007, 95% CI 0.001, 0.013 p=0.023 and 0.007, 95% CI 0.001, 0.013 p=0.02 respectively in temporal, insular and occipital lobes. There was no difference by phenotype detected in the parietal lobe.

Table 4.7 Distribution of CGM lesions (Numbers)

	Frontal			**Temporal**			**Parietal**			**Occipital**			**Insular**		
	IC	LC	JC	IC	LC	JC	IC	LC	JC	IC	LC	JC	IC	LC	JC
RR (n30)	8.8 (4.7)	4.3 (5.6)	2.7 (3.4)	5.3 (3.9)	3.9 (4.3)	1.2 (1.7)	4.4 (2.7)	1.8 (2.2)	1.4 (2.0)	0.5 (1.0)	0.3 (0.7)	0.3 (0.8)	1.3 (1.2)	0.3 (0.5)	0.1 (0.4)
PP (n15)	5.6 (3.8)	6.3 (8.7)	2.6 (2.8)	3.5 (2.8)	3.0 (2.5)	1.8 (2.3)	1.9 (2.3)	1.8 (2.8)	0.9 (1.4)	0.4 (0.7)	0.3 (1.0)	0.3 (0.5)	1.6 (1.6)	0.2 (0.4)	0.1 (0.4)
SP (n15)	9.5 (5.9)	10.0 (8.7)	6.1 (6.7)	5.2 (3.5)	6.7 (5.0)	2.1 (1.8)	3.1 (2.9)	3.7 (4.0)	2.2 (3.3)	0.9 (1.8)	0.6 (0.8)	0.7 (1.5)	2.3 (2.1)	0.9 (0.7)	0.5 (0.8)

Mean (SD) for CGM lesion numbers (IC /LC/JC) across the five lobes and three phenotypes studied.

5 Accrual and evolution of cortical grey matter lesions : observations on follow up

5.1 Abstract

Background

Cortical grey matter (CGM) lesions are seen in all subtypes of MS. Histopathological studies have demonstrated more extensive GM demyelination in people with progressive compared with relapsing-remitting (RR) MS, suggesting a potential role in the accumulation of irreversible disability. CGM demyelination has also been linked to meningeal inflammation, providing a plausible mechanism for this to occur independently of white matter (WM) demyelination. It is not known how GM demyelination progresses and whether or not it is related to WM damage. Longitudinal data studying the evolution of CGM lesions over time is limited. The objective of this study was to study the accrual and evolution of CGM lesions in people with multiple sclerosis (MS).

Methods

49 MS patients (27 RR, 22 SP (secondary progressive)) and 13 healthy controls were scanned using a Philips 3T Achieva System. PSIR

(0.5x0.5x2mm) and PDT2 (1x1x3mm) scans were acquired at baseline and at follow-up and at least one year apart. Follow-up images were registered to baseline, using the niftyreg package using an affine transformation. On baseline scans, CGM lesion were marked and sub classified as intracortical (IC, only involving cortex) and leucocortical (LC, mixed GM-WM lesions); WM lesions touching but not entering the cortex were classified as juxtacortical (JC) lesions. On follow up scans, new IC, LC and JC lesions were identified, and any change in classification of lesions previously observed was noted. New WM lesions were counted on follow-up PDT2 scans.

Results

At baseline, the mean (standard deviation) number of lesions noted was 20.3 (9.8) IC, 16.4 (11.9) LC and 7.6 (9.3) JC in RR and 23.5 (10.6), 23.1 (15.3) and 11.6 (12.5) in SPMS . Controls had 1.6 (2.6) LC lesions and no IC or JC lesions. After a mean(SD) follow up period of 20.5 (7.2) months, accrual of new IC lesions was greater in in SP 2.4 (2.6) than RR 1.1 (2.0); Mann-Whitney p =0.050. No new JC / LC lesions were noted on follow up. RRMS had a greater increase in the number of WM lesions than SPMS (1 (1.9)) vs. 0.5 (0.7); Mann-Whitney p=0.690, and WM lesion number did not change in controls on follow-up. More IC lesions became LC lesions in SP (2.1 (1.9)) than RRMS (1.7 (1.5));Mann-Whitney p=0.564) The change in CGM (IC and LC) lesion load did not correlate with the change in WM lesion load.

Conclusion

People with SPMS have a significantly greater accrual of cortical demyelinating lesions than those with RRMS, but increase in IC lesions counts are offset by the extension of IC lesions into WM (so becoming LC) with time. This process appears to be mostly independent of WM lesion accrual.

5.2 Introduction

Histopathological (Seewann et al. 2009; Bø, Vedeler, Nyland, Trapp & Mørk 2003b; Bø & Bø 2009; Geurts, Pouwels, et al. 2005b) and radiological studies have clearly established that grey matter (GM) pathology is substantial and clinically relevant in multiple sclerosis (MS). CGM lesions are seen across all subtypes of MS, (Seewann et al. 2009; Geurts, Pouwels, et al. 2005b; Nelson, Poonawalla, Hou, Huang, Wolinsky & Narayana 2007a; Bø, Vedeler, Nyland, Trapp & Mørk 2003b) and even in patients with clinically isolated syndrome (CIS) (Filippi, Rocca, Calabrese, Sormani, Rinaldi, Perini, Comi & Gallo 2010b). Histopathology studies have demonstrated more extensive GM demyelination in people with progressive compared with relapsing-remitting (RR) MS (Kutzelnigg et al. 2005a). Longitudinal studies have demonstrated only moderate correlations between change in white matter T2 MRI lesion load and accumulation of disability, (Fisniku et al. 2008) and it is plausible that increasing cortical demyelination has a potential role in the accumulation of irreversible disability. Longitudinal studies could also provide an understanding the relationship between accumulating CGM lesions and disability (Roosendaal et al. 2009).

The investigation of CGM lesion accrual is difficult using histopathological studies; tissue is usually only available at only one time point, and comes either from people with early atypical clinico-radiological presentations that necessitate biopsy, or from post mortem (i.e. those with clinically advanced MS). Furthermore, pathological examination of the whole brain is also very time consuming. Until recently, it has been challenging to visualise CGM lesions even using magnetic resonance imaging

(MRI).However, with the development of sequences such as the double inversion recovery (Geurts et al. 2011; Turetschek et al. 1998) this has become possible. Using DIR, the accrual of CGM lesions over time has also been studied (Filippi & Rocca 2010; Roosendaal et al. 2009; Calabrese et al. 2008). In a 3 year follow up in patients with RRMS (Calabrese, Rocca, et al. 2010c), CGM lesions were found to accumulate with time and were associated with progression of disability (Calabrese et al. 2013)

As demonstrated in my cross sectional comparisons of PSIR and DIR (Chapters 2, 3). PSIR is both more sensitive and more accurate in depicting and quantifying CGM lesions. I thus decided to investigate new CGM lesions using follow-up PSIR. With the improved identification and classification of lesions using PSIR, (Chapters 2 and 3) (Nelson, Poonawalla, Hou, Huang, Wolinsky & Narayana 2007a) I aim to study the accrual of CGM lesions, their location (IC, LC) ,whether they evolve differently in RRMS and SPMS, and whether they enlarge to involve more than one region over time, e.g., IC to LC or JC to LC, or whether they become smaller, i.e., changing from LC to IC.

Patients with the common relapse onset form of MS can become disabled either through the effect of relapses with incomplete recovery during the relapsing remitting phase of the disease or from a steady accumulation of deficits in the secondary progressive phase. The accrual of new CGM lesions in relapse-onset MS has not been previously studied using PSIR.

Another question is whether the accumulation of new CGM lesions is correlated with or independent of the accumulation of new WM lesions. In comparison to WM lesions, CGM lesions have less inflammation and are characterized by demyelination together with axonal transection and

apoptosis leading to neuronal loss (Peterson et al. 2001). CGM demyelination has also been linked with meningeal inflammation, providing a plausible mechanism for this to occur independently of WM demyelination. However, it is not known how closely GM and WM lesion accrual is linked in life, and whether or not the relationship differs between RR and progressive forms of MS. This is important, as current disease modifying treatments for MS have been proven to reduce WM lesion accrual, but there is limited data on their effect against GM demyelination, although in a recent study, using DIR, natalizumab has been found to suppress the accrual of CGM lesions and cortical atrophy (Rinaldi et al. 2011; Rinaldi et al. 2012).

Using a high resolution PSIR sequence, I studied the accrual and evolution of CGM lesions, how this related to changes in WM lesions, and to relapses and the progression of disability. My focus was on RRMS and SPMS and a comparison of the two, in order to investigate the hypothesis that SPMS is characterized by a greater accumulation of CGM lesions.

5.3 Methods

Sixty five people participated in this follow up phase of the project. All patients gave a written informed consent for the study. Scans from 3 patients with RRMS had to be excluded due to motion artefacts. Amongst the remaining 62 people, 49 had clinically definite MS (Lublin et al. 1996; Polman et al. 2011) (27 RR(relapsing remitting), 22 SP(secondary progressive)) and 13 participants were healthy controls (See Table 5.1). Of these, 12 RR, 8 SP and 11 healthy controls had also taken part in the first part of this study (Chapter 2). A detailed clinical history was recorded and neurological examination conducted. Disease duration, number of relapses since baseline

visit and any change of disease modifying therapy were recorded. Expanded disability status scale score was estimated (Kurtzke 1983) and the MS Functional composite with its three components (9-hole peg test, 25 foot timed walk and Paced auditory serial addition test (PASAT)(Polman & Rudick 2010; Smith 1982)was also assessed.

PSIR (0.5x0.5x2mm) and PDT2 (1x1x3mm) scans were acquired on a 3T Philips Achieva system, at baseline and at follow-up, at least one year apart (Chapter 2 ; Table 2.2). No upgrades to the hardware were undertaken during this period. Follow-up images were registered to baseline, using the niftyreg package using an affine transformation. On baseline scans, CGM lesion were marked as intracortical (IC) and leucocortical (LC); WM lesions touching but not entering the cortex were marked as juxtacortical (JC) lesions. On follow up scans, new IC/LC/JC lesions were counted, and the anatomical location of old CGM lesions was studied to observe any change (e.g. IC to LC). New WM lesions were also counted on follow up PDT2 scans. All lesion marking was done under the supervision of an experienced neuroradiologist (TY). Marking of all scans was done blind to clinical details.

The statistical analysis was performed using SPSS version 21 (SPSS, Chicago, IL, USA). Differences in demographic and clinical variables between RR and SPMS were examined using independent sample t-tests. Since lesion numbers tend not to be normally distributed, changes in lesion numbers were compared between patient groups using the Mann-Whitney test. Spearman rank correlation was used to investigate associations between demographic / clinical variables and both lesion accrual and cross-sectional lesion variables (baseline and follow-up).

5.4 Results

5.4.1 Demographics

The demographic and clinical details are recorded in Table 5.1. As expected people with SPMS were older (53.4(7.3) vs. 41.7 (10.9); p<0.001) and had longer disease durations than RRMS (25.4(10) vs. 13.1(9.5); p<0.001). The mean (SD) follow up period for patients with RR was 20.8 (7.6) months and for SP (18.0 (6.2) months) was not significantly different. The median EDSS for patients with RRMS was 1 at baseline (range 1 – 6) and 1.5 (range 1-6) at follow up and for SPMS this was 6.5 (range 4-8.5) at both time points.

In the interval between baseline and follow up, disease modifying therapy was started for one patient and stepped up/changed in 5 patients (3 switched to natalizumab and 1 each changed to interferon beta-1a taken once weekly intramuscularly and thrice weekly subcutaneously).

5.4.2 Baseline comparisons

At baseline, the mean (SD) number of lesions was 20.3 (9.8) IC, 16.4 (11.9) LC and 7.7 (9.3) JC in RR and 23.5 (10.6), 23.1 (15.3) and 11.6 (12.5) respectively in SP. Controls had 1.6 (2.5) LC lesions and no IC/JC lesions. The baseline IC (p=0.487), LC (p=0.124) and JC (p=0.183) lesion numbers did not differ significantly between the RR and SP groups

Table 5.1 Participant demographics

	Age (years)	M:F	EDSS Median (Range) (baseline)	Disease Duration* (years)	Interval (months)	EDSS Median (Range) (baseline)
C (n=13)	35.1 (12.2)	6 : 7			24.0 (6.8)	
RR (n=27)	41.7 (11.0)	9: 13	1 (1 - 6.0)	13.1 (9.5)	20.8 (7.6)	1.5 (1 - 6.0)
SP (n=22)	53.4 (7.3)	6 : 21	6.5 (4 -8.5)	25.4 (10)	18.0 (6.2)	6.5 (4 -8.5)

(Mean (SD) indicated below for C-Controls, SP=secondary progressive; RR=relapsing remitting MS)

Table 5.2 Number of CGM lesions seen at baseline and follow up

At Baseline				**At Follow Up**					
MS type	IC	LC	JC	New IC	IC to LC	Net Gain of IC	IC	LC	JC
C (n13)	Nil	1.6 (2.6)	Nil	Nil	Nil	Nil	Nil	1.6 (2.6)	Nil
RR (n27)	20.3 (9.9)	16.4 (11.9)	7.7 (9.3)	1.1 (2.0)	1.7 (1.5)	-0.2 (2.2)	19.7 (10.2)	18.2 (12.2)	7.7 (9.2)
SP (n22)	23.5 (10.6)	23.1 (15.3)	11.6 (12.5)	2.4 (2.6)	2.1 (1.9)	0.8 (2.9)	23.7(10.6)	25.2 (16.0)	11.6 (12.5)

(Mean (SD) indicated below for C-Controls, SP=secondary progressive; RR=relapsing remitting MS)

5.4.3 Changes over time

As at baseline, even on follow up, SP had a higher number of lesions than RR (Table 5.2) but there was no significant difference between the two groups for IC (p=0.181) , LC (p=0.097) and JC (p=0.183) lesions. All lesions seen at baseline were still visible at follow up. The accrual of new IC lesions (Figure 5.1,Figure 5.2) was greater in patients with SPMS (2.4 (2.6)) than RRMS (1.1 (2.0)) (Mann-Whitney p =0.050). No new JC or LC lesions were seen. RRMS had a greater (though non-significant) increase in the number of WM lesions than SPMS 1 (1.9) vs. 0.5(0.7); Mann-Whitney p=0.690). WM lesion number did not change in controls on follow-up.

The mean number of lesions converting from IC (at baseline) to LC lesions (at follow up) was (non-significantly) greater in SPMS than RRMS (2.1 (1.9) vs. 1.7 (1.5); Mann-Whitney p=0.564). (See Figure 5.3). No JC lesion was seen to change to LC. No new CGM lesions were seen in controls.

The mean (SD) number of new IC and WM lesions in people who had a relapse during the study period (n=11), were 1.2 (1.5) and 1.6 (2.7) respectively and the mean number of lesions changing from IC to LC was 1.6 (1.1). In the remaining 38 patients who did not have a relapse, the figures were 1.8 (2.5), 0.5 (0.8) and 1.9 (1.8) respectively. These numbers did not differ between the relapsing and non-relapsing group. (Mann-Whitney p=0.959, 0.912 and 0.419 for differences in new IC, new WM and IC to LC lesions respectively).

To account for the difference in duration of follow up in the RR and SP groups, the ratio of number of lesions to time lag was compared. Taking into account duration in this way had no effect on the difference in WM (Mann-

Whitney p=0.806) or IC (Mann-Whitney p=0.07) lesion accrual.

The absolute number of IC lesions at follow-up have to be interpreted allowing for both accrual of lesions and conversion of IC to LC lesions. These data show a small net increase of IC lesions in SP +0.3(2.6) vs. a small net decrease in RR -0.6(2.2) (Mann-Whitney p=0.426).

5.4.4 Relationship between new IC lesions, new WM lesions and lesions evolving from IC to LC

In the entire patient group, there was no correlation between the number of new WM(0.7(1.5)) lesions and mean number of lesions converting from IC to LC (1.8(1.6)) (r=0.242; p=0.093). There was also no correlation between number of new WM lesions and number of new IC lesions 1.6(2.3) (r=-0.166; p=0.254). When examining this for individual phenotypes, this observation did not change. (RR: new WM and IC to LC r=0.248 p=0.212, new WM to new IC r=-0.244 p=0.219; SP: new WM and IC to LC r=0.248, p=0.265, new WM to new IC r =-0.150, p=0.504).

5.4.5 Correlations between lesion counts and clinical features (Table 5.3)

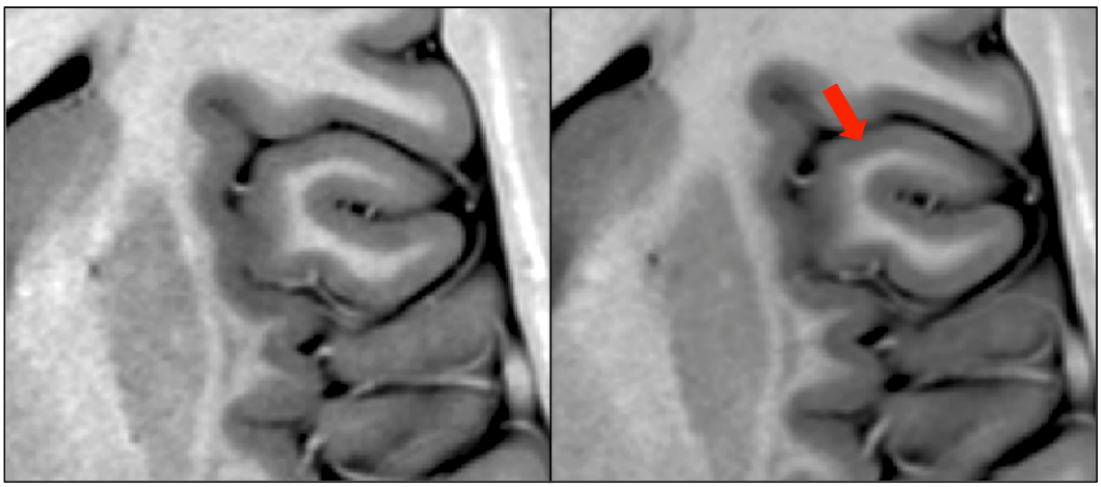
On reviewing the clinical history 11 people (8 RR, 3 SP) had experienced a relapse during the follow up period, 9 of whom received treatment with steroids (3 oral 5 intravenous, and one received both). Following the relapse, there was a 0.5 step increase in EDSS for 2 patients (RR) and an increase of 1 step in one RR patient. Considering the whole cohort (n=49) 17 patients had a worsening of EDSS (11RR and 6SP). Amongst these 17, 15 patients had a 0.5 EDSS step increase on follow up and 2 patients worsened by 1 step. One patient was seen to transition from

RR to SP during the follow up.

In patients who had a worsening of EDSS (n=17) the mean (SD) increase in IC and WM lesions was 1.4 (2.7) and 0.8 (1.7) respectively, and the number changing from IC to LC was 1.4(1.3). In patients where there was no change in EDSS (n=32), the increase in IC and WM lesions was 1.8 (2.2) and 0.6(1.5) respectively; the number changing from IC to LC was 2.1(1.7). Differences between these two groups of EDSS worsening, and non-worsening patients, for new IC (p=0.145), new WM (p=0.471) and IC to LC (p=0.172) were not significant.

Performance on the PASAT correlated significantly with LC and JC lesion counts at baseline (r=0.477 p0.001, r=0.436 p=0.001) and follow-up (r=0.425, p=0.002; r=0.436, p=0.002; Table 5.3). EDSS also correlated modestly with number of LC and JC lesions at baseline (r=0.307 p=0.032, r=0.286,p=0.046); however, at follow up the EDSS did not correlate significantly with JC lesions, while a modest correlation with LC lesion number remained (r=0.0321, p=0.02). There was no significant correlation of either the number of new lesions or the number of lesions changing from IC to LC with EDSS or MSFC components at follow up. Age and duration of illness did not correlate with the number of new lesions or change in lesion location.

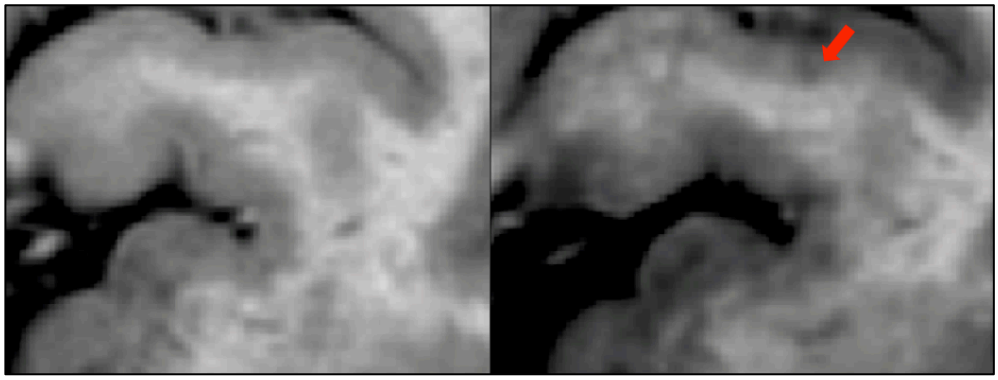
Figure 5.1 New Intracortical (IC) lesion



PSIR images at baseline (left) and follow-up (right) time point

Arrow showing a new IC lesion seen on follow up scan (right) (block arrow)

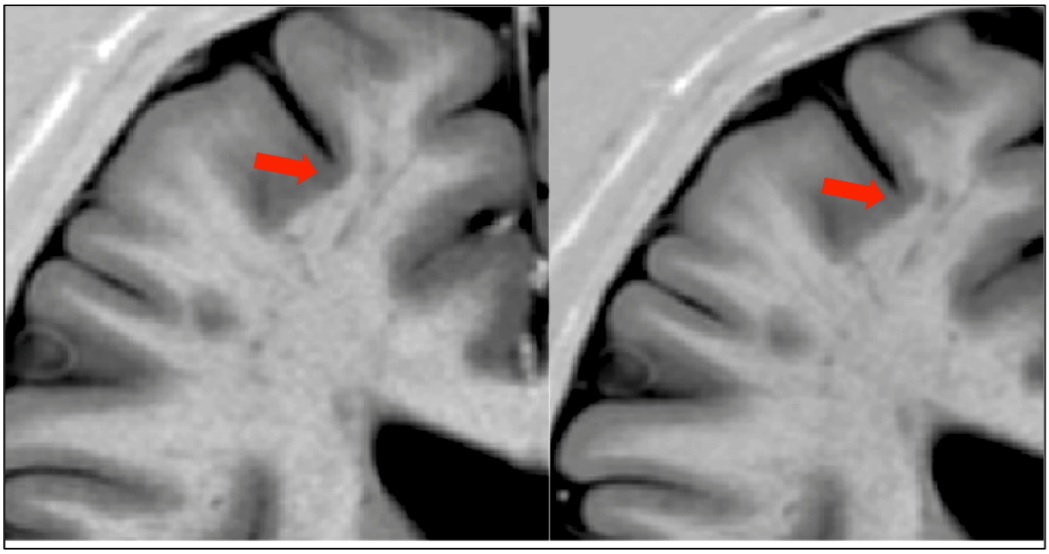
Figure 5.2 New Intracortical (IC) lesion



PSIR images at baseline (left) and follow-up (right) time point

Arrow showing a new IC lesion seen on follow up scan (right) (block arrow)

Figure 5.3 Change in lesion location on follow up



Corresponding PSIR images at baseline (left) and follow-up (right) showing progressive involvement of WM in a IC lesion (at baseline) that then becomes LC on follow up

Table 5.3 Correlations between lesion numbers and demographics / MSFC components

		Baseline			**At Follow Up**					
		IC (B)	LC(B)	JC (B)	IC (F)	LC (F)	JC(F)	New IC	New WM	IC to LC
Age	r	-0.034	-0.084	-0.105	0.056	-0.076	-0.105	0.213	-0.053	-0.050
	p	0.819	0.567	0.474	0.700	0.606	0.474	0.142	0.715	0.731
Baseline EDSS	r	0.003	0.307*	0.286*	0.069	0.320*	0.286*	0.194	0.140	0.121
	p	0.984	0.032	0.047	0.636	0.025	0.047	0.181	0.337	0.406
Disease Duration	r	-0.036	0.130	0.251	-0.001	0.136	0.251	0.048	-0.036	0.037
	p	0.808	0.371	0.081	0.995	0.351	0.081	0.746	0.806	0.802
Timed walk (z-score)*	r	-0.167	-0.182	-0.091	-0.110	0.183	-0.091	0.056	0.286*	-0.028
	p	0.252	0.211	0.530	0.452	0.203	0.530	0.702	0.047	0.847
9HPT (z-score)*	r	-0.237	-0.233	-0.074	-0.224	-0.261	-0.074	-0.074	0.225	-.294*
	p	0.101	0.107	0.616	0.122	0.070	0.616	0.614	0.121	0.040
Pasat (z-score)*	r	-0.151	**0.477**	**0.436**	-0.146	**0.425**	**0.436**	-0.076	0.015	-0.024
	p	0.301	**0.001**	**0.002**	0.318	**0.002**	**0.002**	0.605	0.918	0.737
EDSS *	r	0.082	0.314*	0.273	0.144	0.321*	0.272	0.155	-0.089	0.106
	p	0.578	0.030	0.087	0.330	0.026	0.087	0.292	0.538	0.473

(F) at follow-up ; (B) at baseline ; IC-Intracortical, LC-leucocortical and JC-juxtacortical .**Bold font** indicates p<0.01

5.5 Discussion

In 49 relapse onset patients studied I confirmed – as already reported in Chapters 2 and 3 - that SPMS patients had a higher CGM lesion load than RRMS. In previous data, development of CGM lesions has been reported considering IC, LC and JC together as CGM lesions (Roosendaal et al. 2009) but using PSIR it was possible to demonstrate the accrual at different locations separately. People with SPMS had significantly more new IC lesions than those with RRMS (p=0.050), and showed a non-significant tendency for existing IC lesions become LC more often. The increase in WM lesions was greater in RRMS than SPMS (p=0.690). The accrual of GM lesions appeared to be independent of new WM lesion formation. Modest correlations were noted between LC lesions and baseline EDSS at both baseline and follow-up. In comparison to IC lesions, more robust correlation of LC and JC lesion counts were seen with PASAT. No new CGM or WM lesions were seen in the healthy controls.

The total number of IC lesions at baseline and follow-up were higher in SP than RR, suggesting that focal cortical pathology is more extensive in the SP phase of disease (Roosendaal et al. 2009). Though this is strongly supported by histopathological studies (Bø, Vedeler, Nyland, Trapp & Mørk 2003b; Kutzelnigg et al. 2005a), the majority of cortical demyelination noted histopathologically is subpial. Lesions that I identified as IC may partially be involving the subpial region although in this study, it was not possible to identify lesions involving the subpial region. This is probably because of multiple factors including small size of lesions, proximity to CSF, and a low contrast between demyelinated and non-demyelinated subpial cortex; which

in turn is because the normal density of myelin is lowest in this part of the cortex.

In these data about 10% of IC lesions became LC, with a slightly higher frequency of this evolution seen in SP (2.1(1.9)) than RR (1.7(1.5)). This suggests that expansion of IC lesions to involve the adjacent JC white matter is a not uncommon occurrence in relapse onset MS. However, it was not specific to the SP phase of the disease and therefore does not seem useful in distinguishing the RR and SP phases of MS.

The GM/WM boundary has been reported to be a site of predilection for accrual of CGM lesions in histopathological (Brownell & Hughes 1962) and radiological studies(Nielsen et al. 2013)(Chapter 4). The data in my serial suggest that even during the relapsing remitting phase of disease, there is a shift in pathology towards the GM/WM boundary, with time. Even amongst the 27 RRMS patients, LC lesions increased from 16.4(11.9) to 19.7(10.2) on follow up.

While IC lesions were seen to involve the JC WM and become LC, no de novo appearance of LC lesions was noted during the study period. Further, I saw no lesions converting from JC to LC. Further studies looking at larger cohorts and longer durations of follow up will help ascertain if LC lesions truly represent a pathological process distinct from that of purely WM lesions. The accrual of WM lesions this cohort was greater in RR > SP (though the difference was not significant), with numbers similar to those in other studies. (Calabrese et al. 2008).

The absolute number of IC lesions at follow-up have to be interpreted allowing for both accrual of lesions and conversion of IC to LC lesions. I found

a small net increase of IC lesions in SP vs. a small net decrease in RR (Mann-Whitney p=0.426). As IC lesions did not disappear, the reduction in overall IC counts in RRMS can be attributed to existing IC lesions becoming LC more often than new IC lesions appearing. On the other hand, in SPMS there was a higher number of new IC lesions than existing IC lesions becoming LC.

Unlike my experience with PSIR, in longitudinal studies using DIR, disappearing CGM lesions have been noted on follow-up. It has been hypothesized that only more inflammatory lesions are visible on DIR (Bagnato et al. 2006) and thus have a greater propensity to disappear on follow up (Roosendaal et al. 2009). CGM lesions in themselves have generally less inflammation than WM lesions (Bo1 et al. 2001), and this may reduce the sensitivity of DIR to detect them. It is possible that PSIR is more able to identify CGM lesions beyond the initial inflammatory state. Disappearing lesions could also possibly be due to remyelination (Albert et al. 2007b) which if effective and complete may no longer appear lesional; post-mortem verification of findings using PSIR shall help establish correlations between pathology and radiology.

A comparison of lesion numbers between patients who had relapses (n=11) and those who did not (n=38) suggests that IC lesion accrual and conversion (to LC lesions) continued independently of the relapses. On the other hand, consistent with many previous published studies, patients with relapses had a higher accrual of new WM lesions (1.6(2.7) vs. 0.5 (0.8)).

Studies with larger number of patients and longer duration of follow-up are needed to investigate the evolution of new WM and CGM lesions at

different stages of MS. The overall number of new lesions that I observed were quite small and larger and longer studies would undoubtedly add power in searching for differences in the patters of CGM lesion evolution between RR and SPMS. It may also be useful to separately monitor new CGM in treatment trials: if they develop through mechanisms that are independent of those causing new WM lesions, it can not be assured that treatments that are effective at suppressing WM lesion formation will be as effective against GM lesion accrual, particularly in progressive MS. Although Roosendaal et al. (Roosendaal et al. 2009) reported significant correlations between CGM lesions and WM lesions, in their study, JC lesions, which are essentially WM lesions were also included as CGM. My study using high resolution PSIR scans emphasizes the importance of being able to separate JC lesions from LC and IC lesions in order to accurately determine the relationship between each lesion type and clinical use.

When considering these observations, it should be recalled that even with PSIR it is likely that the majority of GM lesions are still not detected. In particular subpial lesions, that make up the bulk of those identified histopathologically and that have been most closely associated with meningeal inflammation, have not been convincingly seen on PSIR. Longitudinal studies that maybe able to quantify accrual of subpial lesions using ultra-high field scanning (9.4T; (Schmierer et al. 2010) or could help to test the hypothesis relating to the contribution of meningeal inflammation to cortical demyelination.

The one patient who transitioned from RR to SP during the study period, had a disease duration of 17 years. The interval between baseline and

follow-up for this patient was 27 months. No relapses occurred during this period and treatment with beta-interferon had continued though the study period. Though at both time-points this patient was able to walk a distance of 100m with unilateral assistance (EDSS 6), there was a slowing noted, with raw time on the 25TWT test increasing from 10.9 to 15.1 seconds. An accrual of one IC lesion, and a shift of one IC to LC was noted (net gain zero) with no new PDT2 lesions. Though just one case, this case highlights (i) the ongoing cortical demyelination in the absence of relapses and (ii) progression independent of an accrual of WM lesions.

Healthy controls had 3.7(5.7) WM lesions at baseline and 1.6 (2.6) CGM lesions, all of which were LC. There was no accrual of new CGM lesions or change in CGM lesions classification noted in these subjects. Though limited by the small number of controls, this is suggestive of a specificity of my findings of new IC lesions for MS per se although further investigation is warranted with larger group of healthy controls and especially in other neurological conditions that have white matter lesions e.g. migraine, small vessel disease etc.

Correlations with clinical measures and global lesion loads were modest (as in Chapter 2). As reflected in the results of Chapter 4, the regional distribution of lesions based on lobes and anatomical locations improves correlations with cognitive measures. EDSS correlated modestly with both LC and JC lesion counts at baseline; however on follow up there was a loss of this correlation with JC lesions. This may indicate that the involvement of CGM (as a component of LC) lesions continues to contribute to progressive irreversible disability with progression of disease. The more robust correlation

between PASAT and LC and JC lesion counts at both time points is consistent with both cortical and immediately adjacent WM pathology having an influence on attention deficits in MS.

Observations regarding number of IC / LC lesions in RR and SP at both baseline and follow up, are similar to those reported in the cross-sectional study in Chapter 2 and consolidates the original cohort observations, as the cohort reported in this Chapter includes a substantial number of new subjects. This consistency in my observations has probably also been assisted by the use of guidelines that I established for the marking of CGM lesions on PSIR (see Chapter 2). Multicenter studies testing these guidelines are needed so that consensus recommendations can be prepared for use of PSIR, allowing a standardization of methods, observations and results.

The mean duration of follow up in this follow up study was 21 months – and it is possible a longer period of follow up studies will provide an opportunity to extend the present observations and clarify the similarities and differences in CGM lesion evolution that occur in RRMS and SPMS. Primary progressive MS patients are thought to be pathologically different (Montalban et al. 2009; Brück et al. 2002; Lucchinetti & Brück 2004) from relapse onset disease, with a fewer cerebral WM lesions and a relatively more prominent involvement of the spinal cord, including a neurodegenerative component, from the onset. A future study of CGM evolution in the subset of PPMS patients will allow a good comparison with relapse onset MS patients.

In conclusion, I found in this longitudinal study that new CGM lesions appear to develop independently of new WM lesions and to occur relatively more often in SPMS than RRMS whereas new WM lesions appear more often

in RRMS. These observations suggest that detection of new CGM lesions on MRI sequences sensitive to their presence provides additional insights in to disease evolution over and above the detection of new WM lesions on conventional MRI. Suppression of cortical demyelination as an end point in therapeutic trials warrants further investigation, with the potential to control / slow the accumulation of irreversible disability and cognitive dysfunction seen in patients with MS in so far as these functional effects can be related to cortical demyelination.

6 Are grey matter lesions specific to MS? : A comparison with Fabry's disease

6.1 Abstract

Background

The detection and distribution of CGM lesions in MS has been studied extensively and discussed in the preceding chapters. Detection of white matter lesions is an important feature for establishing the diagnosis of MS. However, white matter lesions are commonly see in other conditions such as small vessel disease, migraine and neuroinflammatory conditions such as sarcoidosis. Fabry's (also known as Anderson-Fabry) disease is a X linked inherited disorder and findings similar to small vessel disease have been reported on neuroimaging. The occurrence and frequency of CGM lesions may help differentiate from MS when the diagnosis is suspected but not confirmed.

Aim

To use high resolution PSIR at 3T, to study CGM lesions in Fabry's disease and investigate if this can be used to as an aid in the differential diagnosis of MS.

Methods

PSIR and PDT2 scans were acquired for 15 patients with Fabry's disease. These were compared with the participants in the earlier study

described in Chapter 2. (30 RRMS, 15 SPMS, 15PPMS and 30 healthy controls). CGM lesions were marked using JIM version 6.0 (Xinapse Systems), as per the guidelines outlined in Chapter 2. Lesions were identified as intracortical (IC), leucocortical (LC) or juxtacortical (JC), and counted separately. Additional note was made of the morphology of lesions. SPSS (version 21, Chicago) was used for the statistical analysis

Results

The mean (SD) number of lesions in the Fabry's disease patients was IC 2.4(1.9), LC 9.6(4.8) and WM 4.0(4.3). No juxtacortical lesions were noted. Within the Fabry's group, there were significantly more LC than IC lesions ($p=0.001$). In comparison to Fabry's, people with MS were found to have a higher number of IC and WM lesions ($p<0.001$). Oval, wedge shaped and curvilinear lesions were noted in both MS and Fabry's and 21.6% of the curvilinear lesions were located along the gyral crown in Fabry's whereas in MS all such lesions were along the sulcal pit.

Conclusion

Cortical lesions (IC and LC) are seen in patients with Fabry's, The frequency of CGM lesions in the Fabry's cohort was more than of healthy controls, but less than that observed in people with MS. IC lesions and a curvilinear morphology are see in both MS and Fabry's.

6.2 Introduction

In the previous chapters I have reported the frequency, distribution, accrual and evolution of CGM lesions in patients with MS and in healthy controls. If these changes are specific for MS then CGM lesions can help us differentiate MS from other conditions with white matter lesions when the diagnosis is suspected but not confirmed. This includes conditions such as small vessel disease (Nichtweiss et al. 2012; Assareh et al. 2010), migraine (Nichtweiss et al. 2012; Colombo et al. 2011; Assareh et al. 2010; Rossato et al. 2010), other headache disorders (Applebee 2012), neuroinflammatory conditions such as sarcoidosis (Fels et al. 2004), lupus (Cikes et al. 2008) , other connective tissue diseases and systemic vasculitides (Schedel et al. 2010).

Grey matter abnormalities in the form of CGM lesions, atrophy and quantitative changes (MTR, DTI measures) have been reported in other neurological conditions. Cortical microinfarcts (van Veluw et al. 2012) in vascular disease have been studied in the context of vascular dementia. In Alzheimer's disease the evolution of disease is associated with the occurrence of CGM lesions which initially occur in the temporal lobe. Epilepsy (Kakita 2013), heat stroke (Fuse et al. 2013) and schizophrenia (Narr et al. 2005; Pol et al. 2001) are some other conditions in which cortical pathology has been observed, in the form of lesions and changes in cortical thickness. The presence of focal CGM lesions may have potential to aid differential diagnosis; the lack of CGM lesions has been reported in neuromyelitis optica (Calabrese et al. 2012) and this observation needs further investigation

across other neuroinflammatory conditions.

Fabry's (also known as Anderson-Fabry) disease is a X linked inherited disorder. It was first described as a condition affecting the skin, in 1898. A defect in GLA gene (Kint 1970) leads to deficiency of alpha galactosidase A, which is a functionally active enzyme. Hemizygotes with the condition have no detectable activity of this enzyme. In female carriers an intermediate level of enzyme activity is seen. This results in a disorder of lysosomal storage with cellular accumulation of glycosphingolipids with terminal alpha galactosyl moieties such as globotriaosylceramide (ceramide trihexoside, gal-gal-gal-glc-ceramide), most notably in blood vessels (Paavilainen et al. 2013), and galabiosylceramide (gal–gal–ceramide)(Prineas 1997) in various tissues. Prominent pathological features in the nervous system include deposition of glycosphingolipids in the endothelial and smooth muscle cells of bloods vessels. This leads to a vasculopathy leading to cerebral ischemia / infarcts. Deposits of glycosphingolipid (Møller & Jensen 2007) are seen in the Schwann cells and deramide trihexoside deposits have been observed in the blood vessels of entorhinal cortex; these are also seen in the brain stem, amygdala, hypothalamus, spinal cord and ganglia, leptomeningeal cells (deVeber et al. 1992) and astrocytes (Sobel et al. 2008) however, studies have reported no storage of glycolipids in the cerebral cortex (Kahn 1973).

Clinical manifestations of Fabry's disease can vary and multi-system affections are commonly seen. Neurological symptoms affecting both central and peripheral nervous systems (Ginsberg et al. 2007) are seen in about 72% patients (Hoffmann 2009) and is often seen early in disease (Mehta et al.

2004). The central nervous system is involved as a result of a cerebral vasculopathy affecting both small and larger vessels; transient ischemia attacks and cerebral infarcts are often seen. Peripheral neuropathy results in intermittent paraesthesia, neuropathic pain and acroparaesthesia, which can affect the whole body (Hoffmann 2009). In addition, cortical presentations including seizures and cognitive impairment have been reported in Fabry's disease.

Neuro-radiological characteristics of Fabry's disease are varied. Lesions indicative of small vessel disease are seen in patients with Fabry's disease(Prineas 1997). Other findings include infarcts involving grey and white matter, T2 signal abnormalities in the periventricular region and T1 hyperintensities in the pulvinar region. Areas of abnormal signal (hypointense on T1w scans) in the sub-cortical white matter have also been reported in patients with Fabry's (Ginsberg et al. 2007). It is not clear whether the WM lesions represent areas of ischemia or demyelination (Ginsberg et al. 2007). The accumulation of disseminated white matter lesions (Ginsberg et al. 2007) in about 80% patients (male and female) (Buechner et al. 2008), combined with intermittent sensory symptoms sometimes leads to a fulfillment of the McDonald criteria (Polman et al. 2005; Polman et al. 2011; McDonald et al. 2001) for possible/ definite MS (Böttcher et al. 2013). CSF analysis in Fabry's disease may suggest aseptic meningitis (Lidove et al. 2009) and can be interpreted incorrectly. Fabry's disease may thus sometimes be considered in a differential diagnosis of MS (Saip et al. 2007; Böttcher et al. 2013; Solomon & Weinshenker 2013; Callegaro & Kaimen-Maciel 2006).

Global and regional grey matter volumes have not been found to differ

between people with Fabry's, and healthy controls (Paavilainen et al. 2013). Cortical regions that appear normal on conventional scans, have shown a reduction of N-acetyl aspartate on proton MR spectroscopy (Prineas 1997), perhaps due to underlying cerebral ischemia (Tedeschi et al. 1999). Cerebral atrophy has also been reported (Buechner et al. 2008) but the occurrence of CGM lesions has not been explored in radiological studies.

In previous chapters I have reported that in comparison to controls, the occurrence of pure intracortical lesions, and a curvilinear morphology are more frequent in MS patients. In this study I investigated the frequency, subtype (IC, LC and JC) and morphology (curvilinear, oval and wedge-shaped) of CGM lesions in patients with Fabry's disease and explore whether it differed from patients with MS (and healthy controls).

6.3 Methods

PSIR and PDT2 scans had been acquired for 60 patients (30 RRMS, 15 SP and 15PPMS) and 30 healthy controls (Chapter 2). In addition, PSIR scans were acquired for 15 patients with Fabry's disease, who were recruited by colleagues in the Department of Metabolic disease. Acquisition parameters for PSIR and PD/T2 scans were the same as described in earlier Chapters (2,3,4). For this group of patients with Fabry's, FLAIR scans were not available. Demographic distribution of this group of 15 Fabry's patients was also recorded.

Scans for the cohort of 60 MS patients were reviewed. I identified CGM lesions and further examined the marked lesions that had a curvilinear morphology and noted whether they were in the sulcal pit or over the gyral crown (as reported in Chapter 2). For the 15 Fabry's patients, CGM lesions

were marked using JIM version 6.0 (Xinapse Systems), as per the guidelines outlined in Chapter 2. Lesions were identified as intracortical (IC), leucocortical (LC) or juxtacortical (JC), and counted separately. Additional note was made of the morphology of lesions; the number of curvilinear, oval and wedge shaped lesions was counted. For curvilinear lesions I noted whether they stretched along the sulcal pit or over the gyral crown. The number of white matter lesions (non-JC) were counted using PDT2 and PSIR scans. All lesion marking was under in under supervision of two experienced researchers (DC, TY). The appearance of VR spaces in the insular region was also noted. All methods were the same as used for data from MS patients in the preceding chapters (Chapter 2, 3)

SPSS (version 21, Chicago) was used for the statistical analysis. Since lesion numbers tend not to be normally distributed, the following non-parametric tests were used: Wilcoxon signed rank for comparison of differences within subjects; Mann-Whitney for comparison of differences between groups; and Spearman correlation for investigating associations between quantitative variables. A value of $p<0.05$ is reported as significant.

6.4 Results

Demographic details for healthy controls and MS patients have been detailed in Chapter 2, Table 2.1. The fifteen patients with a confirmed diagnosis of Fabry's disease (8 male and 7 female) had a mean (SD) age of 43.3(12.4) years; the diagnosis had been confirmed using genetically confirmed GAL mutations. These subjects were participating in a separate research study involving multiparameter MRI and neuropsychological investigation. For the present analysis of PSIR scans for CGM lesions, no

additional clinical or imaging data was used.

6.4.1 Group of patients with Fabry's disease

Amongst these 15 patients, at least one IC lesion was seen in 12 patients and all patients had at least one LC lesion. The mean (SD) number of lesions per patient (See Table 6.1) in the Fabry's disease patients was IC 2.4(1.9), LC 9.6(4.8) and WM 4.0(4.3). No juxtacortical lesions were noted in any of the patients with Fabry's disease using PSIR scans at 3T. Within the Fabry's group, there were significantly more LC than IC lesions (Wilcoxon signed rank p=0.001).

The mean number of WM lesions on PSIR (4 (4.3)) correlated strongly with the WM lesion count on PDT2 scans (mean=5.6 (5.3)) (Spearman correlation r=0.985; p<0.001). The number of IC, LC and WM lesions did not differ significantly between males and females (all p>0.500). On the PSIR scans, there was no significant correlation between the number of IC lesions and number of WM lesions (r=0.447, p=0.095).

6.4.2 Comparison in lesion numbers : Fabry's vs. MS and vs. Controls

The mean(SD) number of lesions for the MS cohort are detailed in Chapter 2 (

Table 2.4). On comparing the number of lesions between the two groups, MS and Fabry's, people with MS were found to have a higher number of IC and WM (on both PSIR and PDT2) lesions than Fabry's(for all comparisons Mann-Whitney p<0.001). There was no significant difference in number of LC lesions between the MS cohort and Fabry's disease. As

reported above, no JC lesions were seen in Fabry's.

Table 6.1 Distribution of lesions in the study participants

	On PSIR				On PD/T2
	IC	LC	JC	Non JC WM	Non JC WM
Controls n=30	One	0.7 (1.8)	Nil	2.6 (4.4)	3.7 (5.3)
PP N=15	12.3 (8.8)	11.7 (12.7)	6.1 (5.5)	50.4 (31.7)	48.3 (27.6)
SP N=15	21.3 (10)	20.9 (13.7)	11.1 (11.2)	80.5 (40.7)	72.9 (33)
RR N=30	19.5 (9.2)	10.5 (11.4)	5.8 (6.8)	63.4 (57.2)	61.2 (51.2)
Fabry's N=15	2.4 (2.0)	9.6 (4.9)	Nil	4.0 (4.3)	5.7 (5.3)

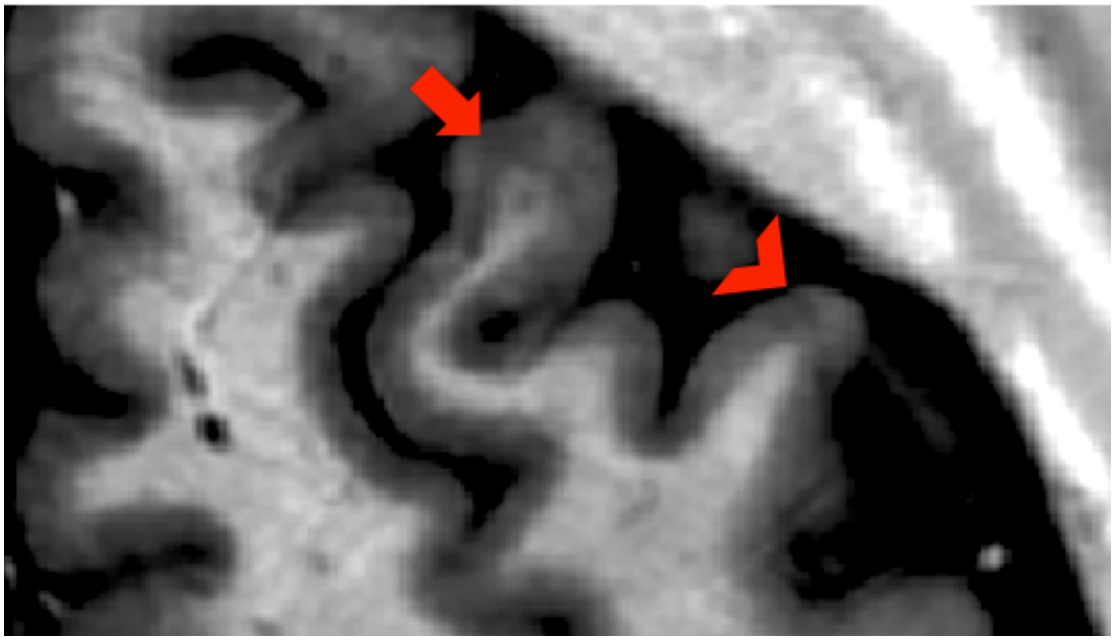
Above data indicates mean (SD) of number of lesions across the study participants

Table 6.2 Comparison of lesion morphology

	Oval lesions	**Curvilinear lesions**	**Wedge shaped lesions**
Fabry's	15/15	15/15	8/15
MS	47/60	51/60	20/60

Numbers above are the number of patients with at least one lesion of the respective morphology

Figure 6.1 Examples of CGM lesions in Fabry's disease



Example of an IC (bold chevron) lesion; the blocked arrow indicates a LC lesion with a curvilinear morphology along the gyral crown

I further anlaysed comparisons with Fabry's by looking at each phenotype of MS separately. The above results remained unchanged on considering patients with primary progressive MS and relapse-onset MS separately versus Fabry's disease. On comparing SPMS with Fabry's disease, the difference in LC lesions was also significant (Mann-Whitney $p=0.011$) (See Figure 6.1, Table 6.1). In comparison to controls, patients with Fabry's disease had a higher number of IC and LC lesions (Mann-Whitney $p<0.001$ for both); WM lesion numbers did not differ significantly between controls and Fabry's disease.

6.4.3 Comparison in lesion morphology : Fabry's vs. MS and vs. Controls

I compared the number of oval, curvilinear and wedge shaped lesions across the participants. Using Fisher's exact test, comparisons of proportions for oval, curvilinear and wedge lesions between the Fabry's and MS cohort

showed no significant differences. (Oval 15/15 (100%) vs. 47/60 (78%) (p=0.059), curvilinear 15/15 (100%) vs. 51/60 (85%) (p=0.190) and wedge 8/15 (53%) vs. 20/60 (33%) (p=0.232)) See Table 6.2.

In comparison to controls, curvilinear lesions were found to be more specific for MS (at least one curvilinear lesion see in 51/60 patients with MS and in 0/ 30 controls, Chapter 2). I studied these further by observing the location of curvilinear lesions as being along the sulcal pit vs. the gyral crown. There is no standard definition to demarcate the sulcal region from the gyrus in imaging studies (Samson et al. 2013). For purposes of this work, I recognised the part of the cortex abutting the dura mater as gyral and the rest as sulcal. In the MS patients, all curvilinear lesions were noted in the sulcus and none in the gyrus. In Fabry's 21.6% of the total curvilinear lesions were located along the gyral crown.

6.5 Discussion

In this study I identified the presence of CGM lesions (IC and LC) in patients with Fabry's disease. Cortical pathology in Fabry's disease was largely at the GM/WM boundary and pure CGM lesions (IC) were considerably less common. The frequency of CGM lesions in the Fabry's cohort was more than the normal population, but less than that observed in people with MS.

Using PSIR at 3T, involvement of both intracortical and leucocortical locations was noted: 2.4 (1.9) IC and 9.6 (6.8) LC lesions were identified. The pathological substrate of these lesions in Fabry's disease is not known. These lesions appear hypointense (low signal, implying long T1) and ischemia leading to cortical infarcts is a possibility. The mechanism of cerebral

ischemia could be due to the underlying vasculopathy (Ginsberg et al. 2007) that causes hypoxia. Hypoxia in turn has been reported causes alterations in the endothelial cells leading to a disruption of the blood brain barrier (Kaur & Ling 2008). In addition hypoxia leads to a derangement of the tight junction proteins and these factors together maybe leading to increased permeability of the blood brain barrier. This is the more likely mechanism for CGM lesions, as opposed to lipid deposition in lepotomeningeal cells (Elleder et al. 1994), which would shorten the T1, causing a hyper-intense appearance on T1 weighted imaging.

No significant difference between lesions numbers was noted in males in comparison to females. Heterozygous females are relatively less symptomatic than affected males but can develop symptoms with increasing age (Prineas 1997); the impact of genotype on CGM lesions would require further genotype details, which were not available in this study and could be explored further in a future study.

In the patients with Fabry's disease, the number of WM lesions on PSIR correlated well with the detection on PDT2 (Spearman's correlation r=0.985; p=0.000). This is similar to the finding in Chapter 2 and re-emphasises the utility of PSIR for detection of WM lesions in addition to being able to CGM. I found a non-significant trend for correlation between IC and WM lesion numbers (r=0.447, p=0.095) in Fabry's disease; investigation of larger cohorts is needed to clarify whether there is indeed a relationship.

My study indicates that the occurrence of IC lesions is not specific to MS alone, although they occurred in higher numbers in MS than in Fabry's disease. Intracortical lesions have been suggested as a diagnostic aid in MS

because of a high specificity (Filippi, Rocca, Calabrese, Sormani, Rinaldi, Perini, Comi & Gallo 2010b; Sinnecker et al. 2012; Calabrese et al. 2012); however my observations in Fabry's disease clearly indicates that such a lesion location is not exclusive to MS. Further investigation for evidence of IC lesions using PSIR-MRI is warranted in other neurological conditions that are considered in the differential diagnosis of MS.

Curvilinear, oval and wedge shaped CGM lesions were all seen in Fabry's disease. In controls I did not observe curvilinear lesions. The relative absence of an intracortical location and a curvilinear morphology was characteristic of controls in comparison to both diseases investigated (MS and Fabry's disease). While 85% patients with MS were found to have a curvilinear lesion, all patients with Fabry's were found to have at least one curvilinear lesion: thus the simple appearance of a curvilinear CGM lesion does not appear specific for MS; however, investigation for this type of lesion in other conditions that enter the MS differential diagnosis would seem warranted.

I compared the location of curvilinear lesions between MS and Fabry's disease and found that while none of these lesions were located over the gyral crown in MS; 21.6% curvilinear lesions were in the gyral crown in Fabry's disease (See Table 6.2). The suclal region showed preferential involvement in patients with MS. CSF flow is likely to be slower in the regions of the sulcus (Samson et al. 2013) and a prolonged exposure to inflammatory triggers, with meningeal inflammation leading to subpial demyelination, has been proposed as a theory for sulcal predilection of CGM MS lesions. The occurrence of gyral crown curvilinear lesions in Fabry's disease may reflect a

different pathogenic mechanism. A sulcal predominance (and gyral sparing) of CGM lesions maybe more characteristic of MS than other disease states but this needs to be investigated in a broader range of conditions that enter the differential diagnosis of MS.

No juxtacortical lesions were noted in patients with Fabry's or in healthy controls. JC lesions were therefore specific for patients with MS in this study. Juxtacortical lesions have been identified as a significant predictor for conversion of CIS to clinically definite MS (Barkhof et al. 1997), providing more information than periventricular lesions, hypointense T1 lesions and the total number of T2 lesions. Their presence has also been correlated with neuropsychological impairment (Lazeron et al. 2000). JC lesions are an important aid in the diagnosis of MS, and have been studied extensively using conventional scans such as FLAIR (Yousry et al. 1997; Herskovits et al. 2001). Juxtacortical lesions are part of the McDonald criteria for dissemination in space (Polman et al. 2011; Polman et al. 2005) and my finding of their absence in controls and Fabry's disease using high resolution PSIR scans further supports their specificity for MS. The use of PSIR provides more accurate classification of JC lesions distinct from LC and IC lesions, and my findings raise the possibility that true JC lesions are more specific for MS than has been suggested in previous work using FLAIR scans where it is likely that some lesions classified as JC were actually LC.

In conclusion, in comparison to MS using high resolution PSIR, patients with Fabry's disease have fewer CGM lesions, involvement of the gyral crown and absence of involvement of the juxtacortical region.

7 Conclusion

The projects undertaken above have aimed to assess (i) the detection and classification of CGM lesions using PSIR, (ii) evaluate the significance of CGM lesions in the clinical setting and (iii) researched the distribution, location and evolution of CGM lesions to help improve the understanding of the pathogenesis of CGM lesions in MS.

7.1 Detection of Cortical grey matter lesions using PSIR

Using a cohort representative of all common MS phenotypes, (RR, SP and PPMS), I first compared the detection of CGM lesions on a standard resolution DIR (1x1x3mm)(Geurts et al. 2011) vs. high resolution PSIR (0.5 x0.5 x 2mm), acquired using Philips 3T Achieva system. The results (Chapter2) indicated that PSIR detects a significantly greater number of CGM lesions (about three times) than DIR (Geurts et al. 2011). In addition to the quantitative improvement (Chapter 2), a qualitative improvement with respect to the anatomical accuracy of lesions (Chapter 3) was also demonstrated with the use of PSIR. Subpial regions could however not be seen using PSIR at 3T. PSIR can be used to study both grey and white matter (Chapters 2 and 5), and has the potential to be included in routine scanning protocols across clinical and research centres.

7.2 Clinical impact of cortical lesions

The clinical impact of CGM lesions includes a potential role in diagnosing MS and differentiating it from other conditions, understanding of differences in MS subtypes, association with cognition and disability.

7.2.1 Diagnosis and differential diagnosis

In this project I attempted to determine if the findings of CGM lesions are specific to patients with MS. Intracortical lesions, were seen only in one control (Chapter 2), and in Fabry's, their occurrence was significantly less compared to MS (Chapter 6). The curvilinear morphology of lesions, while common in MS patients and not seen in controls and was observed in all patients with Fabry's. It is not clear whether the morphology of lesion is indicative of a separate pathological process. In keeping with the role of JC lesions in diagnosing MS, using conventional scans, I also observed that these lesions were specific for MS. I noted no JC lesions in controls or in people with Fabry's disease.

7.2.2 Can cortical lesions help differentiate phenotypes?

In this work the data suggests that people with secondary progressive disease have a greater number of CGM lesions at all locations –IC, LC and JC (Chapters 2, 4 and 5) and can be seen early in disease. The shortest disease duration amongst all patients in this study was 1 year in a patient with RRMS; 9 IC and 2 LC, JC each were identified in this patient. No people with clinically isolated syndrome were recruited in this study, however literature suggests that CGM lesions are seen even in people with CIS (Filippi et al. 2010b). One healthy control was noted to have an isolated IC lesion in my data, and should be followed up in time, considering the possibility that this may represent a radiologically isolated syndrome. Regional distribution of lesions, across lobes was not found to be significantly different between MS phenotypes.

7.2.3 Impact on Cognition and disability

When comparing total CGM lesion counts, correlations between clinical measures and CGM lesions counts were limited to the relapse-onset group; the most consistent correlations were PSIR LC counts with the Symbol Digit Modality Test (Chapter 2). I expected correlations to improve on comparing regional CGM lesions, measured as volumes. Results in chapter 4 indicate that both CGM and juxta-cortical lesions are associated with cognitive defects; the associations varying with different lobar regions. Functional localization of cognitive dysfunction has to be interpreted with caution allowing for differences between tasks that are more network driven as compared to others.

7.2.4 Treatment

Current disease modifying therapy (DMT) is aimed at reducing the accrual of WM lesions and number of clinical relapses. During the follow up period, patients with relapses had a higher accrual of WM lesions; Conversely, IC lesion accrual and conversion (to LC) was independent of the relapses (Chapter 5). These data suggest that the influence of DMT on CGM demyelination cannot be presumed to be the same as that on WM, and merits further investigation. If these new CGM lesions represent 'cognitive relapses' then it is important to recognise the accrual of new lesions and use this an end point in future clinical trials.

7.3 Relevance to pathogenesis

The pathology of GM MS lesions has been suggested to be location dependent (Brink et al. 2005; Bø & Bø 2009) and the location differences

represent differences in pathogenesis of lesions at different sites (Reynolds et al. 2011; Kutzelnigg et al. 2005a); e.g. complement activation causes oligodendrocyte and myelin damage and is seen to be low in pure CGM lesions in comparison to LC and pure WM lesions.

The data reported in this thesis suggest heterogeneity in the number of CGM lesions at different locations and across different lobes and clinical MS phenotypes. With progression of disease a major proportion of CGM lesions are found to be located at the GM/WM boundary (Chapters 2, 4 and 5) and accrual of IC lesions continues. It is possible that lesions that I have classified as IC may represent lesions that have subpial component and then extend into the thickness of the cortex, and are influenced by the meningeal inflammation. On follow up no new LC lesions were noted and no JC lesions were seen to transform to LC. The accrual of LC lesions was due to IC lesions extending to involve the subcortical WM, suggesting that their pathogenesis is linked to subpial and IC pathology, possibly remaining separate from WM triggers.

There were no significant correlations between the accrual of new WM lesions and development of new IC lesions (Chapter 5). IC lesions thus appear to increase independent of the rate of new inflammatory WM lesions, consistent with the concept that intracortical demyelination continues with an intact blood-brain barrier and minimal inflammation (Reynolds et al. 2011), possibly representing a compartmentalisation of pathogenic processes with progression of disease (Frischer et al. 2009). Furthermore, the number of relapses did not impact the accrual of CGM lesions (Chapter 5) suggesting greater neurodegeneration (vs. neuroinflammation) in cortical pathology

(Reynolds et al. 2011). Recent studies have reported the origin of CGM lesions as being outward from a central vein (Gaitan et al. 2012) In this data the proximity of lesions to blood vessels has not been studied and should be planned in future studies.

7.4 Limitations and future direction

In this study subpial lesions could not be identified, using 3T imaging. Limited detection of subpial lesions may not matter should CGM lesion detection per se prove useful for diagnosis, but may be relevant when monitoring evolving pathology, especially in progressive MS. In the longitudinal study in this thesis, patients with primary progressive disease have not been included and this comparison with relapse-onset MS should be studied in the future to help understand difference in pathogenesis. In this thesis, regional differences in distribution of CGM lesions have been explored by lobe; by being more selective in regions of comparison - e.g. cingulate gyrus, hippocampus - a stronger association between CGM lesions and clinical and cognitive measures may become apparent.

Further studies are needed to reproduce these observations using PSIR for the study of CGM in MS. A comparison across centres will allow standardisation of marking criteria enabling the formation of consensus recommendations for detection of CGM lesions. Combined radiology and pathology studies will improve the understanding of the significance of CGM lesions, their frequency and morphology. To elucidate the specificity of findings in MS, a comparison with other neurological conditions is also warranted.

In conclusion, CGM lesions are important in the pathogenesis of MS and Phase sensitive inversion recovery, a T1w MRI sequence, can improve detection and classification of CGM lesions, in clinically acceptable times. CGM lesions are seen across all subtypes of MS, being more common in progressive disease, and have a potential role to help with diagnosis of MS when it is suspected but not confirmed. The distribution, accrual and evolution of lesions provides insights into the pathogenesis of MS and the contribution of CGM lesions to neurological and cognitive impairment. The use of CGM lesions as endpoints in clinical trials needs to be explored further.

8 Bibliography

Absinta, M. et al., 2011. Cortical lesions in children with multiple sclerosis. *Neurology*, 76(10), pp.910–913.

Absinta, M. et al. 2013. Seven-tesla phase imaging of acute multiple sclerosis lesions: A New Window into the Inflammatory Process. *Annals of neurology*, 74(5), pp.669-678.

Achiron, A. et al., 2003. Cognitive impairment in probable multiple sclerosis. *Journal of Neurology, Neurosurgery & Psychiatry*, 74(4), pp.443–446.

Albert, M. et al., 2007a. Extensive cortical remyelination in patients with chronic multiple sclerosis. *Brain pathology (Zurich, Switzerland)*, 17(2), pp.129–138.

Albert, M. et al., 2007b. Extensive Cortical Remyelination in Patients with Chronic Multiple Sclerosis. *Brain Pathology*, 17(2), pp.129–138.

Allen, I.V. et al. 2001. Pathological abnormalities in the normal-appearing white matter in multiple sclerosis. *Neurological Sciences*, 22(2), pp.141–144.

Amato, M.P. et al., 2004. Neocortical volume decrease in relapsing-remitting MS patients with mild cognitive impairment. *Neurology*, 63(1), pp.89–93.

Andersson, M. et al., 1994. Cerebrospinal fluid in the diagnosis of multiple sclerosis: a consensus report. *Journal of Neurology, Neurosurgery & Psychiatry*, 57(8), pp.897–902.

Applebee, A., 2012. The clinical overlap of multiple sclerosis and headache. *Headache*, 52 Suppl 2, pp.111–116.

Archibald, C.J. et al., 2000. Information Processing Efficiency in Patients with Multiple Sclerosis. *Journal of Clinical and Experimental Neuropsychology (Neuropsychology, Development and Cognition: Section A)*, 22(5), pp.686–701.

Assareh, A. et al., 2010. The Genetics of White Matter Lesions. *CNS Neuroscience & Therapeutics*, 17(5), pp.525–540.

Bagnato, F. et al., 2006. In vivo detection of cortical plaques by MR imaging in patients with multiple sclerosis. *AJNR. American journal of neuroradiology*, 27(10), pp.2161–2167.

Bakshi, R., 2003. Fatigue associated with multiple sclerosis: diagnosis, impact and management. *Multiple sclerosis (Houndmills, Basingstoke, England)*, 9(3), pp.219–227.

Bakshi, R. et al., 2008. MRI in multiple sclerosis: current status and future prospects. *Lancet neurology*, 7(7), pp.615–625.

Barkhof, F. et al., 1997. Comparison of MRI criteria at first presentation to predict conversion to clinically definite multiple sclerosis. *Brain : a journal of neurology*, 120 (Pt 11), pp.2059–2069.

Barkhof, F., 2002a. The clinico-radiological paradox in multiple sclerosis revisited. *Current opinion in neurology*, 15(3), p.239-245.

Barkhof, F., 2002b. Whole-brain T 1 -relaxation time measurements in multiple sclerosis. *Journal of neurology*, 249(10), pp.1451–1452.

Barkhof, F. et al., 2003. Remyelinated lesions in multiple sclerosis: magnetic resonance image appearance. *Archives of neurology*, 60(8), pp.1073–1081.

Barkhof, F. et al., 2009a. Imaging outcomes for neuroprotection and repair in multiple sclerosis trials. *Nature Publishing Group*, 5(5), pp.256–266.

Barkhof, F. et al., 2009. Multiple sclerosis: MRI - the perfect surrogate marker for multiple sclerosis? *Nature reviews. Neurology*, 5(4), pp.182–183.

Barnett, M.H. et al., 2004. Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. *Annals of neurology*, 55(4), pp.458–468.

Bender, B. et al., 2010. Double inversion recovery: impact of incidental magnetic transfer effects on optimal inversion times. *Investigative radiology*, 45(4), pp.196–201.

Benedict, R.H. et al., 2012. Reliability and equivalence of alternate forms for the Symbol Digit Modalities Test: implications for multiple sclerosis clinical trials. *Multiple sclerosis*, 18(9), pp. 1320-1325.

Bjartmar, C. et al., 2001. Axonal loss in normal-appearing white matter in a patient with acute MS. *Neurology*, 57(7), pp.1248–1252.

Bjartmar, C. et al., 2003. Axonal loss in the pathology of MS: consequences for understanding the progressive phase of the disease. *Journal of the neurological sciences*, 206(2), pp.165–171.

Bø, L. et al. , 2001. Intracortical Multiple Sclerosis Lesions Are Not Associated With T Lymphocyte Infiltration. *Acta Neurologica Scandinavica, 103(5), pp.323-333.*

Bø, L., et al., 2003a. Intracortical multiple sclerosis lesions are not associated with increased lymphocyte infiltration. *Multiple sclerosis (Houndmills, Basingstoke, England)*, 9(4), pp.323–331.

Bø, L., et al., 2003b. Subpial demyelination in the cerebral cortex of multiple sclerosis patients. *Journal of neuropathology and experimental neurology*,

62(7), pp.723–732.

Bø, L., et al., 2006a. Grey matter pathology in multiple sclerosis. *Acta neurologica Scandinavica*, 113(s183), pp.48–50.

Bø, L., et al., 2006b. Grey matter pathology in multiple sclerosis. *Acta neurologica Scandinavica. Supplementum*, 183(s183), pp.48–50.

Bø, L. et al., 2009. The histopathology of grey matter demyelination in multiple sclerosis. *Acta neurologica Scandinavica*, 120(189), pp.51–57.

Bobholz, J.A. et al., 2003. Cognitive dysfunction in multiple sclerosis: a review of recent developments. *Current opinion in neurology*, 16(3), pp.283–288.

Borrello, J.A., et al., 1990. Regional phase correction of inversion-recovery MR images. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*, 14(1), pp.56–67.

Böttcher, T. et al., 2013. Fabry disease - underestimated in the differential diagnosis of multiple sclerosis? *PloS one*, 8(8), p.e71894.

Boulby, PA. et al. 2004 Optimized interleaved whole-brain 3D Double inversion recovery for imaging the neocortex. *Magnetic Resonance in Medicine* (51) pp 1181-86.

Bramow S. et al., 2010. Demyelination versus remyelination in progressive multiple sclerosis. Brain (133) pp. 2983-2998.

Brink BP. et al., 2005. The pathology of multiple sclerosis is location dependent : no significant complement activation is detected in purely cortical lesions. *Journal of neuropathology and experimental neurology*(64) pp 147-155.

Brink, B.P. et al., 2005. The Pathology of Multiple Sclerosis Is Location-Dependent: No Significant Complement Activation Is Detected in Purely Cortical Lesions. *Journal of neuropathology and experimental neurology*, 64(2), p.147.

Brownell, B. et al., 1962. The distribution of plaques in the cerebrum in multiple sclerosis. *Journal of Neurology, Neurosurgery & Psychiatry*, 25, pp.315–320.

Brück, W. et al., 2002. The pathology of primary progressive multiple sclerosis. *Multiple sclerosis (Houndmills, Basingstoke, England)*, 8(2), pp.93–97.

Buckner, R.L. et al., 2005. Molecular, structural, and functional characterization of Alzheimer's disease: evidence for a relationship between default activity, amyloid, and memory. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 25(34), pp.7709–7717.

Buechner, S. et al., 2008. Central nervous system involvement in Anderson-Fabry disease: a clinical and MRI retrospective study. *Journal of Neurology, Neurosurgery & Psychiatry*, 79(11), pp.1249–1254.

Burgess, P.W. et al., 1997 *The Hayling and Brixton Tests*, Thames Valley Test company/ Pearson Assessment, Bury St Edmunds, Suffolk.

Calabrese, M. et al., 2007. Detection of Cortical Inflammatory Lesions by Double Inversion Recovery Magnetic Resonance Imaging in Patients With Multiple Sclerosis. *Archives Neurology*, 64(10), pp.1416–1422.

Calabrese, M. et al., 2008. Morphology and evolution of cortical lesions in multiple sclerosis. A longitudinal MRI study. *NeuroImage*, 42(4), pp.1324–1328.

Calabrese, M. et al., 2009. Cortical lesions and atrophy associated with cognitive impairment in relapsing-remitting multiple sclerosis. *Archives of neurology*, 66(9), pp.1144–1150.

Calabrese, M. et al., 2010a. Imaging distribution and frequency of cortical lesions in patients with multiple sclerosis. *Neurology*, 75(14), pp.1234–1240.

Calabrese, M. et al., 2010b. Cortical lesions in multiple sclerosis. *Nature Publishing Group*, 6(8), pp.438–444.

Calabrese, M. et al., 2010c. A 3-year magnetic resonance imaging study of cortical lesions in relapse-onset multiple sclerosis. *Annals of neurology*, 67(3), pp.376–383.

Calabrese, M. et al., 2011a. Cortical pathology and cognitive impairment in multiple sclerosis. *Expert review of neurotherapeutics*, 11(3), pp.425–432.

Calabrese, M. et al., 2011b. The puzzle of multiple sclerosis: gray matter finds its place. *Expert review of neurotherapeutics*, 11(11), pp.1565–1568.

Calabrese, M. et al., 2011c. Cortical diffusion-tensor imaging abnormalities in multiple sclerosis: a 3-year longitudinal study. *Radiology*, 261(3), pp.891–898.

Calabrese, M. et al., 2012. No MRI evidence of cortical lesions in neuromyelitis optica. *Neurology*, 79(16), pp.1671-1676.

Calabrese, M. et al., 2013. The changing clinical course of multiple sclerosis: a matter of gray matter. *Annals of neurology*, 74(1), pp.76–83.

Calabresi, P.A., 2011. Inflammation in multiple sclerosis--sorting out the gray matter. *The New England journal of medicine*, 365(23), pp.2231–2233.

Callegaro, D. et al., 2006. Fabry's disease as a differential diagnosis of MS. *Int MS Journal, 13(1), pp.27-30.*

Ceccarelli, A. et al., 2008. A voxel-based morphometry study of grey matter loss in MS patients with different clinical phenotypes. *NeuroImage*, 42(1), pp.315–322.

Ceccarelli, A. et al., 2012a. MRI in multiple sclerosis: a review of the current literature. *Current opinion in neurology*, 25(4), pp. 402-409.

Ceccarelli, A. et al., 2012b. The Impact of Lesion In-Painting and Registration Methods on Voxel-Based Morphometry in Detecting Regional Cerebral Gray Matter Atrophy in Multiple Sclerosis. *American Journal of Neuroradiology*, 33(8), pp.1579-1585.

Cerasa, A. et al., 2012. Cerebellar-parietal dysfunctions in multiple sclerosis patients with cerebellar signs. *Experimental neurology*, 237(2), pp.418–426.

Chard, D.T. et al., 2002. Brain metabolite changes in cortical grey and normal-appearing white matter in clinically early relapsing-remitting multiple sclerosis. *Brain : a journal of neurology*, 125(10), pp.2342–2352.

Chard, D.T. et al., 2009. Grey matter pathology in clinically early multiple sclerosis: evidence from magnetic resonance imaging. *Journal of the neurological sciences*, 282(1-2), pp.5–11.

Chard, D.T. et al., 2010. Reducing the impact of white matter lesions on automated measures of brain gray and white matter volumes. *Journal of magnetic resonance imaging : JMRI*, 32(1), pp.223–228.

Charil, A. et al., 2006. MRI and the diagnosis of multiple sclerosis: expanding the concept of “no better explanation.” *The Lancet Neurology*, 5(10), pp.841–852.

Chen, X. et al., 2012. Iron deposition of the deep grey matter in patients with multiple sclerosis and neuromyelitis optica: A control quantitative study by 3D-enhanced susceptibility-weighted angiography (ESWAN). *European journal of radiology*, 81(4), pp.e633–e639.

Ciccarelli, O. et al., 2012. MS cortical lesions on double inversion recovery MRI: Few but true. *Neurology*, 78(5), pp.296–297.

Cifelli, A. et al., 2002. Thalamic neurodegeneration in multiple sclerosis. *Annals of neurology*, 52(5), pp.650–653.

Cikes, N. et al., 2008. Non-MS autoimmune demyelination. *Clinical neurology and neurosurgery*, 110(9), pp.905–912.

Colombo, B. et al., 2011. Brain white matter lesions in migraine: what's the meaning? *Neurological Sciences*, 32 Suppl 1, pp.S37–40.

Compston, A., 1999. The pathological anatomy of the lesion in multiple sclerosis. *Brain research bulletin*, 50(5-6), pp.463–464.

Compston, A. et al., 2006. *McAlpines's Multiple Sclerosis* Fourth edition, Churchill Livingstone Elsevier, Section 4, pp.447-669.

Confavreux, C. et al., 2014. The clinical course of multiple sclerosis. *Handbook of clinical neurology / edited by P.J. Vinken and G.W. Bruyn*, 122, pp.343–369.

Crawford, M.P. et al., 2004. High prevalence of autoreactive, neuroantigen-specific CD8+ T cells in multiple sclerosis revealed by novel flow cytometric assay. *Blood*, 103(11), pp.4222–4231.

Cutter, G.R. et al., 1999. Development of a multiple sclerosis functional composite as a clinical trial outcome measure. *Brain : a journal of neurology*, 122 (Pt 5), pp.871–882.

Davies, G.R. et al., 2004. Evidence for grey matter MTR abnormality in minimally disabled patients with early relapsing-remitting multiple sclerosis. *Journal of Neurology, Neurosurgery & Psychiatry*, 75(7), pp.998–1002.

Dawson, J., 1916. The histology of disseminated sclerosis. *Trans. R. Soc. Edinburgh*, 50, pp.517–740.

de Graaf, W.L. et al., 2012. Lesion detection at seven Tesla in multiple sclerosis using magnetisation prepared 3D-FLAIR and 3D-DIR. *European Radiology*, 22(1), pp.221–231.

DeLuca, G.C. et al., 2006. The contribution of demyelination to axonal loss in multiple sclerosis. *Brain : a journal of neurology*, 129(Pt 6), pp.1507–1516.

Deshmukh, V.A. et al., 2013. A regenerative approach to the treatment of multiple sclerosis. *Nature*, 502(7471), pp.327–332.

deVeber, G.A. et al., 1992. Fabry disease: immunocytochemical characterization of neuronal involvement. *Annals of neurology*, 31(4), pp.409–415.

Disanto, G. et al., 2012. Multiple sclerosis: risk factors and their interactions. *CNS & neurological disorders drug targets*, 11(5), pp.545–555.

Dusankova, J.B. et al., 2012. Cross cultural validation of the Minimal Assessment of Cognitive Function in Multiple Sclerosis (MACFIMS) and the Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS). *The Clinical neuropsychologist*, 26(7), pp.1186–1200.

Ebers, G.C. et al., 2008. Natural history of MS. *European journal of neurology : the official journal of the European Federation of Neurological Societies*, 15(9), pp.881–882.

Elleder, M. et al., 1994. Leptomeningeal lipid storage patterns in Fabry disease. *Acta neuropathologica*, 88(6), pp.579–582.

Ellwardt, E. et al., 2014. Molecular mechanims linking neuroinflammation and neurodegeneration in MS. *Experimental neurology*, pp.1–30.

Fels, C. et al., 2004. Neurosarcoidosis: findings in MRI. *Clinical imaging*, 28(3), pp.166–169.

Fernandes de Abreu, D.A.,= et al., 2011. Seasonal, gestational and postnatal influences on multiple sclerosis: the beneficial role of a vitamin D supplementation during early life. *Journal of the neurological sciences*, 311(1-2), pp.64–68.

Filippi, M. et al., 2010. MR Imaging of Gray Matter Involvement in Multiple Sclerosis: Implications for Understanding Disease Pathophysiology and Monitoring Treatment Efficacy. *American Journal of Neuroradiology*, 31(7), pp.1171–1177.

Filippi, M. et al., 2010a. The contribution of MRI in assessing cognitive impairment in multiple sclerosis. *Neurology*, 75(23), pp.2121–2128.

Filippi, M. et al., 2010b. Intracortical lesions: Relevance for new MRI diagnostic criteria for multiple sclerosis. *Neurology*, 75(22), pp.1988–1994.

Filippi, M. et al., 2011. Magnetic resonance techniques in multiple sclerosis: the present and the future. *Archives of neurology*, 68(12), pp.1514–1520.

Filippi, M. et al., 2012. Association between pathological and MRI findings in multiple sclerosis. *The Lancet Neurology*, 11(4), pp.349–360.

Filippi, M. et al., 2013. Ultra-high-field MR imaging in multiple sclerosis. *Journal of Neurology, Neurosurgery & Psychiatry*, 85(1), pp.60-66.

Fischer, J.S. et al., 1999. The Multiple Sclerosis Functional Composite measure (MSFC): an integrated approach to MS clinical outcome assessment. *Multiple Sclerosis*, 5(4), pp.244–250.

Fisniku, L.K. et al., 2008. Disability and T2 MRI lesions: a 20-year follow-up of patients with relapse onset of multiple sclerosis. *Brain : a journal of neurology*, 131(3), pp.808–817.

Fox, R.J. et al., 2005. Brain atrophy and magnetisation transfer ratio following methylprednisolone in multiple sclerosis: short-term changes and long-term implications. *Multiple sclerosis (Houndmills, Basingstoke, England)*, 11(2), pp.140–145.

Frischer, J.M. et al., 2009. The relation between inflammation and neurodegeneration in multiple sclerosis brains. *Brain : a journal of neurology*, 132(5), pp.1175–1189.

Frohman, E. et al., 2006. Multiple Sclerosis - The plaque and its pathogenesis. *New England Journal of Medicine*, 354(9), pp.942-955.

Fuse, A. et al., 2013. Reversible focal cerebral cortical lesions in a patient with

heat stroke. *Internal medicine (Tokyo, Japan)*, 52(3), pp.377–380.

Gaitan, M.I. et al., 2012. Initial investigation of the blood-brain barrier in MS lesions at 7 tesla. *Multiple sclerosis,*19(8), pp.1068-1073.

Gandhi, R. et al., 2012. Chapter 1: Disease Pathogenesis. In *Multiple Sclerosis: Diagnosis and Therapy*. John Wiley & Sons, Ltd. pp 3 - 26.

Garach, R.M. et al., 2004. Robust phase sensitive inversion recovery imaging using a Markov random field model. *Conference proceedings: Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE Engineering in Medicine and Biology Society. Conference*, 2, pp.1569–1572.

Gelineau-Morel, R. et al., 2011. The effect of hypointense white matter lesions on automated gray matter segmentation in multiple sclerosis. *Human brain mapping*, 33(12), pp.2802-2814.

Geurts, J.J.G. et al., 2005a. Cortical Lesions in Multiple Sclerosis: Combined Postmortem MR Imaging and Histopathology. *American journal of Neuroradiology,* 26(3), pp. 572 -577.

Geurts, J.J.G. et al., 2005b. Intracortical Lesions in Multiple Sclerosis: Improved Detection with 3D Double Inversion-Recovery MR Imaging. *Radiology*, 236(1), pp.254–260.

Geurts, J.J.G. et al., 2008. Grey matter pathology in multiple sclerosis. *The Lancet Neurology*, 7(9), pp.841–851.

Geurts, J.J.G. et al., 2008a. Does high-field MR imaging improve cortical lesion detection in multiple sclerosis? *Journal of neurology*, 255(2), pp.183–191.

Geurts, J.J.G. et al., 2011. Consensus recommendations for MS cortical lesion scoring using double inversion recovery MRI. *Neurology*, 76(5), pp.418–424.

Gilmore C.P. et al. 2009 Regional variations in the extent and pattern of grey matter demyelination in multiple sclerosis : a comparison between the cerebral cortex , cerebellar cortex , deep grey matter nuclei and the spinal cord. *Journal of Neurology , Neurosurgery and Psychiatry* 80(2) pp182-187.

Ginsberg, L. et al., 2007. Magnetic resonance imaging changes in Fabry disease. *Acta Paediatrica*, 95, pp.57–62.

Giorgio, A. et al., 2010. Cognition in multiple sclerosis: relevance of lesions, brain atrophy and proton MR spectroscopy. *Neurological Sciences*, 31(Suppl 2), pp.S245–8.

Giorgio, A. et al., 2011. Cortical lesions in radiologically isolated syndrome. *Neurology*, 77(21), pp.1896–1899.

Gonzalez, C.F. et al., 1994. Distributional patterns of multiple sclerosis brain lesions. Magnetic resonance imaging--clinical correlation. *Journal of neuroimaging : official journal of the American Society of Neuroimaging*, 4(4), pp.188–195.

Goris, A., 2012. Comment: The HLA region in multiple sclerosis. *Neurology*, 79(6) , pp.544-544

Granberg, T. et al., 2012. Radiologically isolated syndrome - incidental magnetic resonance imaging findings suggestive of multiple sclerosis, a systematic review. *Multiple sclerosis,* 19(3), pp.271-280.

Grant, I. et al., 1984. Deficient learning and memory in early and middle phases of multiple sclerosis. *Journal of Neurology, Neurosurgery & Psychiatry*, 47(3), pp.250–255.

Graumann, U. et al., 2003. Molecular Changes in Normal Appearing White Matter in Multiple Sclerosis are Characteristic of Neuroprotective Mechanisms Against Hypoxic Insult. *Brain Pathology*, 13(4), pp.554–573.

Griffin, C.M. et al., 2002. The relationship between lesion and normal appearing brain tissue abnormalities in early relapsing remitting multiple sclerosis. *Journal of neurology*, 249(2), pp.193–199.

Guttmann, C.R. et al., 1995. The evolution of multiple sclerosis lesions on serial MR. *AJNR. American journal of neuroradiology*, 16(7), pp.1481–1491.

Guttmann, C.R.G. et al., 2006. Can MRI reveal phenotypes of multiple sclerosis? *Magnetic resonance imaging*, 24(4), pp.475–481.

Hagemeier, J. et al., 2012. Iron deposition in multiple sclerosis lesions measured by susceptibility-weighted imaging filtered phase: A case control study. *Journal of magnetic resonance imaging : JMRI*, 36(1), pp.73–83.

Haider, L. et al., 2014. Multiple sclerosis deep grey matter: the relation between demyelination, neurodegeneration, inflammation and iron. *Journal of Neurology, Neurosurgery & Psychiatry*, pii: jnnp-2014-307712, pp.1-10.

Haller, S. et al., 2009. Magnetic resonance imaging techniques in white matter disease: potentials and limitations. *Topics in magnetic resonance imaging: TMRI*, 20(6), pp.301–312.

Herskovits, E.H. et al., 2001. Accuracy for detection of simulated lesions: comparison of fluid-attenuated inversion-recovery, proton density--weighted, and T2-weighted synthetic brain MR imaging. *AJR. American journal of roentgenology*, 176(5), pp.1313–1318.

Hoffmann, B., 2009. Fabry disease: recent advances in pathology, diagnosis, treatment and monitoring. *Orphanet Journal of Rare Diseases,* 4(1), pp.

1-9

Hou, P. et al., 2005. Phase-Sensitive T1 Inversion Recovery Imaging: A Time-Efficient Interleaved Technique for Improved Tissue Contrast in Neuroimaging. *American journal of Neuroradiology*, 26(6), pp.1432-1438.

Hulst, H.E. et al., 2013. Cognitive impairment in MS: Impact of white matter integrity, gray matter volume, and lesions. *Neurology*, 80(11), pp.1025–1032.

IMSGC et al., 2007. Risk alleles for multiple sclerosis identified by a genome wide study. *The New England journal of medicine*, 357(9), pp.851–862.

Johnson, G. et al., 1999. 2D multislice and 3D MRI sequences are often equally sensitive. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*, 41(4), pp.824–828.

Jongen, P.J. et al., 2012. Cognitive impairment in multiple sclerosis. *Minerva medica*, 103(2), pp.73–96.

Kacar, K. et al., 2011. Overcoming the clinical-MR imaging paradox of multiple sclerosis: MR imaging data assessed with a random forest approach. *American Journal of Neuroradiology*, 32(11), pp.2098–2102.

Kahn, P.,1973. Anderson-Fabry disease: a histopathological study of three cases with observations on the mechanism of production of pain. *Journal of Neurology, Neurosurgery & Psychiatry*, 36(6), pp.1053–1062.

Kakita, A., 2013. Surgical pathologic features of cerebral cortical lesions taken from 600 patients with intractable epilepsy. *Brain & development*, 35(8), pp.793–801.

Kampman, M.T. et al., 2012. Effect of vitamin D3 supplementation on relapses, disease progression, and measures of function in persons with multiple sclerosis: exploratory outcomes from a double-blind randomised controlled trial. *Multiple sclerosis (Houndmills, Basingstoke, England)*, 18(8), pp.1144–1151.

Kapoor, R. et al., 2010. Lamotrigine for neuroprotection in secondary progressive multiple sclerosis: a randomised, double-blind, placebo-controlled, parallel-group trial. *Lancet neurology*, 9(7), pp.681–688.

Kaur, C. et al., 2008. Blood Brain Barrier in Hypoxic-Ischemic Conditions. *Current Neurovascular Research*, 5(1), pp.71–81.

Kemppinen, A. et al., 2011. Genome-wide association studies in multiple sclerosis: lessons and future prospects. *Briefings in functional genomics*, 10(2), pp.61–70.

Kidd, D. et al., 1999. Cortical lesions in multiple sclerosis. *Brain : a journal of neurology*, 122(1), pp.17–26.

Kilsdonk, I.D. et al., 2013. Multicontrast MR Imaging at 7T in Multiple Sclerosis: Highest Lesion Detection in Cortical Gray Matter with 3D-FLAIR. *American Journal of Neuroradiology*, 34(4), pp.791–796.

Kilsdonk, I.D. et al., 2011. 2010 revisions to McDonald criteria for diagnosis of multiple sclerosis: impact of 3-Tesla magnetic resonance imaging. *Annals of neurology*, 70(1), pp.182–183.

Kint, J.A., 1970. Fabry's disease: alpha-galactosidase deficiency. *Science*, 167(3922), pp.1268-1269.

Kleinschmidt-Demasters, B.K. et al., 2012. Update on PML and PML-IRIS Occurring in Multiple Sclerosis Patients Treated With Natalizumab. *Journal of neuropathology and experimental neurology*, 71(7), pp.604–617.

Kurtzke, J.F., 1983. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology*, 33(11), pp.1444–1452.

Kutzelnigg, A. et al., 2005a. Cortical demyelination and diffuse white matter injury in multiple sclerosis. *Brain : a journal of neurology*, 128(Pt 11), pp.2705–2712.

Kutzelnigg, A. et al., 2005b. Cortical lesions and brain atrophy in MS. *Journal of the neurological sciences*, 233(1-2), pp.55–59.

Kutzelnigg, A. et al., 2006. Cortical demyelination in multiple sclerosis: a substrate for cognitive deficits? *Journal of the neurological sciences*, 245(1-2), pp.123–126.

Kutzelnigg, A. et al., 2007. Widespread demyelination in the cerebellar cortex in multiple sclerosis. *Brain pathology (Zurich, Switzerland)*, 17(1), pp.38–44.

Lassmann, H. et al., 2007. The Immunopathology of Multiple Sclerosis: An Overview. *Brain Pathology*, 17(2), pp.210–218.

Lassmann, H. et al., 2008. Cortical demyelination in CNS inflammatory demyelinating diseases. *Neurology*, 70(5), pp.332–333.

Lassmann, H., 2008a. Mechanisms of inflammation induced tissue injury in multiple sclerosis. *Journal of the neurological sciences*, 274(1-2), pp.45–47.

Lassmann, H., 2008b. The Pathologic Substrate of Magnetic Resonance Alterations in Multiple Sclerosis. *Neuroimaging Clinics of North America*, 18(4), pp.563–576.

Lassmann, H., et al., 2012. Progressive multiple sclerosis: pathology and pathogenesis. *Nature Publishing Group*, 8(11), pp.647–656.

Lassmann, H., 2014. Mechanisms of white matter damage in multiple

sclerosis. *Glia,* 62(11), pp 1816-1830.

Lauer, K., 1995. Environmental associations with the risk of multiple sclerosis: the contribution of ecological studies. *Acta neurologica Scandinavica. Supplementum*, 161, pp.77–88.

Lazeron, R.H. et al., 2000. Neuropsychological impairment in multiple sclerosis patients: the role of (juxta)cortical lesion on FLAIR. *Multiple Sclerosis*, 6(4), pp.280–285.

Leary, S., et al., 2009. *Queen Square Textbook of Neurology*, John Wiley & Sons, Ltd. Chapter 10, pp. 412-447.

Lezak, M.D. et al., 2012. *Neuropsychological Assessment* 5 ed., Oxford University Press.

Li, Q. et al., 2011. Double inversion recovery magnetic resonance imaging at 3 T: diagnostic value in hippocampal sclerosis. *Journal of computer assisted tomography*, 35(2), pp.290–293.

Lidove, O. et al., 2009. Aseptic meningitis and ischaemic stroke in Fabry disease. *International journal of clinical practice*, 63(11), pp.1663–1667.

Mehta A. et al., 2004. Fabry disease defined: baseline clinical manifestations of 366 patients in the Fabry Outcome Survey. *Europiean journal of clinical investigation,* 34 (3), pp.236-242.

Lublin, F.D. et al.,1996. Defining the clinical course of multiple sclerosis: results of an international survey. National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis. *Neurology*, 46(4), pp.907–911.

Lucchinetti, C.F. et al., 1996. Distinct patterns of multiple sclerosis pathology indicates heterogeneity on pathogenesis. *Brain pathology (Zurich, Switzerland)*, 6(3), pp.259–274.

Lucchinetti, C. et al., 2000. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Annals of Neurology,* 47(6), pp.707-717.

Lucchinetti, C. et al., 2004. The pathology of primary progressive multiple sclerosis. *Multiple Sclerosis*, 10 Suppl 1, pp.S23–30.

Lucchinetti, C.F. et al., 2011. Inflammatory Cortical Demyelination in Early Multiple Sclerosis. *The New England journal of medicine*, 365(23), pp.2188–2197.

Lumsden, C.E., 1970. The neuropahtology of multiple sclerosis. In Handbook of Clinical Neurology, Vol 9, pp. 217-309.

Lynch, S.G. et al., 2010. Evaluating processing speed in multiple sclerosis: a comparison of two rapid serial processing measures. *The Clinical*

neuropsychologist, 24(6), pp.963–976.

Ma, J., 2005. Multislice and multicoil phase-sensitive inversion-recovery imaging. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*, 53(4), pp.904–910.

Mackenzie, I.S. et al., 2014. Incidence and prevalence of multiple sclerosis in the UK 1990-2010: a descriptive study in the General Practice Research Database. *Journal of Neurology, Neurosurgery & Psychiatry*, 85(1), pp.76–84.

Magliozzi, R. et al., 2006. Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. *Brain : a journal of neurology*, 130(4), pp.1089–1104.

Magliozzi, R. et al., 2007. Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. *Brain : a journal of neurology*, 130(Pt 4), pp.1089–1104.

Magliozzi, R. et al., 2010. A Gradient of neuronal loss and meningeal inflammation in multiple sclerosis. *Annals of neurology*, 68(4), pp.477–493.

Mainero, C. et al., 2009. In vivo imaging of cortical pathology in multiple sclerosis using ultra-high field MRI. *Neurology* ,73(9), pp.41-48.

Maldjian, J.A. et al., 2003. An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *NeuroImage*, 19(3), pp.1233–1239.

Martinelli, V. et al., 2014. Vitamin D levels and risk of multiple sclerosis in patients with clinically isolated syndromes. *Multiple Sclerosis Journal*, 20(2), pp.147–155.

McDonald, W.I. et al., 2001. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Annals of neurology*, 50(1), pp.121–127.

McRobbie, D.W. et al., 2003. *MRI From Picture to Proton*, Cambridge University Press, 2nd Edition, Part A, pp.30-167.

McVeigh, E.R. et al. , 1986. Phase and Sensitivity of Receiver Coils in Magnetic-Resonance-Imaging. *Medical physics*, 13(6), pp.806–814.

Mike, A. et al., 2011. Identification and Clinical Impact of Multiple Sclerosis Cortical Lesions as Assessed by Routine 3T MR Imaging. *American Journal of Neuroradiology*, 32(3), pp.515–521.

Miki, Y. et al., 1998. Isolated U-fiber involvement in MS: preliminary

observations. *Neurology*, 50(5), pp.1301–1306.

Miller, D.H. et al., 2002. Measurement of atrophy in multiple sclerosis: pathological basis, methodological aspects and clinical relevance. *Brain : a journal of neurology*, 125(Pt 8), pp.1676–1695.

Miller, D. et al., 2005. Clinically isolated syndromes suggestive of multiple sclerosis, part 2: non-conventional MRI, recovery processes, and management. *Lancet neurology*, 4(6), pp.341–348.

Miller, D.H. et al., 2012. Clinically isolated syndromes. *Lancet neurology*, 11(2), pp.157–169.

Mistry, N. et al., 2011. Focal multiple sclerosis lesions abound in 'normal appearing white matter'. *Multiple sclerosis (Houndmills, Basingstoke, England)*, 17(11), pp.1313–1323.

Modat, M. et al., 2010. Fast free-form deformation using graphics processing units. *Computer methods and programs in biomedicine*, 98(3), pp.278–284.

Montalban, X. et al., 2009. Primary progressive multiple sclerosis diagnostic criteria: a reappraisal. *Multiple sclerosis (Houndmills, Basingstoke, England)*, 15(12), pp.1459–1465.

Montalban, X. et al., 2010. MRI criteria for MS in patients with clinically isolated syndromes. *Neurology*, 74(5), pp.427–434.

Mueller, S.G. et al., 2010. Influences of lobar gray matter and white matter lesion load on cognition and mood. *Psychiatry Research: Neuroimaging*, 181(2), pp.90–96.

Muhlert, N. et al., 2013. The grey matter correlates of impaired decision-making in multiple sclerosis. *Journal of neurology, neurosurgery and neuropsychiatry,* p.jnnp-2014-308169, pp.1-7.

Møller, A.T. et al., 2007. Neurological manifestations in Fabry's disease. *Nature Clinical Practice Neurology*, 3(2), pp.95–106.

Myhr, K.M. et al., 2001. Disability and prognosis in multiple sclerosis: demographic and clinical variables important for the ability to walk and awarding of disability pension. *Multiple Sclerosis*, 7(1), pp.59–65.

Narr, K.L. et al., 2005. Mapping Cortical Thickness and Gray Matter Concentration in First Episode Schizophrenia. *Cerebral Cortex*, 15(6), pp.708–719.

Neema, M. et al., 2009. Deep Gray Matter Involvement on Brain MRI Scans Is Associated with Clinical Progression in Multiple Sclerosis. *Journal of neuroimaging : official journal of the American Society of Neuroimaging*, 19(1), pp.3–8.

Nelson, F. et al., 2007a. Improved Identification of Intracortical Lesions in Multiple Sclerosis with Phase-Sensitive Inversion Recovery in Combination with Fast Double Inversion Recovery MR Imaging. *American Journal of Neuroradiology*, 28(9), pp.1645–1649.

Nelson, F. et al., 2008. 3D MPRAGE improves classification of cortical lesions in multiple sclerosis. *Multiple sclerosis*, 14(9), pp.1214–1219.

Nelson, F. et al., 2011. Intracortical lesions by 3T magnetic resonance imaging and correlation with cognitive impairment in multiple sclerosis. *Multiple sclerosis*, 17(9), pp.1122–1129.

Nichtweiss, M. et al., 2012. White matter lesions and vascular cognitive impairment: part 1: typical and unusual causes. *Clinical neuroradiology*, 22(3), pp.193–210.

Nielsen, A.S. et al., 2013. Contribution of cortical lesion subtypes at 7T MRI to physical and cognitive performance in MS. *Neurology*, 81(7), pp.641-649.

Nielsen, A.S. et al., 2011. Focal cortical lesion detection in multiple sclerosis: 3 tesla DIR versus 7 tesla FLASH-T2*. *Journal of magnetic resonance imaging : JMRI*, 35(3), pp.537–542.

Paavilainen, T. et al., 2013. Diffusion tensor imaging and brain volumetry in Fabry disease patients. *Neuroradiology*, 55(5), pp.551–558.

Papadopoulou, A. et al., 2013. Contribution of cortical and white matter lesions to cognitive impairment in multiple sclerosis. *Multiple sclerosis, 19(10), pp.1290-1296.*

Patzold, U. et al., 1982. Course of multiple sclerosis. First results of a prospective study carried out of 102 MS patients from 1976-1980. *Acta neurologica Scandinavica*, 65(4), pp.248–266.

Pelvig, D.P. et al., 2008. Neocortical glial cell numbers in human brains. *Neurobiology of aging*, 29(11), pp.1754–1762.

Peterson, J.W. et al., 2001. Transected neurites, apoptotic neurons, and reduced inflammation in cortical multiple sclerosis lesions. *Annals of neurology*, 50(3), pp.389–400.

Pirko, I. et al., 2007. Gray matter involvement in multiple sclerosis. *Neurology*, 68(9), pp.634–642.

Pitt, D. et al., 2010. Imaging Cortical Lesions in Multiple Sclerosis With Ultra–High-Field Magnetic Resonance Imaging. *Archives of neurology*, 67(7), pp.812–818.

Pol, H.E.H. et al., 2001. Focal Gray Matter Density Changes in Schizophrenia. *Archives of General Psychiatry*, 58(12), pp.1118–1125.

Polman, C.H. et al., 2005. Diagnostic criteria for multiple sclerosis: 2005

revisions to the “McDonald Criteria.” *Annals of neurology*, 58(6), pp.840–846.

Polman, C.H. et al., 2010. The multiple sclerosis functional composite: a clinically meaningful measure of disability. *Neurology*, 74 Suppl 3, pp.S8–15.

Polman, C.H. et al., 2011. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Annals of neurology*, 69(2), pp.292–302.

Popescu, B.F.G. et al., 2011. A case of multiple sclerosis presenting with inflammatory cortical demyelination. *Neurology*, 76(20), pp.1705–1710.

Popescu, B.F.G. et al., 2012a. Meningeal and cortical grey matter pathology in multiple sclerosis. *BMC neurology*, 12(1), pp.1-8.

Popescu, B.F.G. et al., 2012b. Pathology of demyelinating diseases. *Annual review of pathology*, 7(1), pp.185–217.

Popescu, B.F.G. et al., 2013. Pathology of Multiple Sclerosis: Where Do We Stand? *CONTINUUM: Lifelong Learning in Neurology*, 19(4, Multiple Sclerosis), pp.901-921.

Poser, C.M. et al., 1983. New diagnostic criteria for multiple sclerosis: Guidelines for research protocols. *Annals of neurology*, 13(3), pp.227–231.

Prineas, J.W. et al., 1993. Multiple sclerosis. Pathology of recurrent lesions. *Brain : a journal of neurology*, 116 (Pt 3), pp.681–693.

Prineas J.W. et al., 1997. Demyelinating diseases In:*Greenfields neuropathology*, 2 (1997); pp.471-550.

Pucci, E. et al., 2011. Natalizumab for relapsing remitting multiple sclerosis (review). *Cochrane Database of Systematic Reviews, Issue 10, pp* 1-99.

Raine, C.S., 1994. The Dale E. McFarlin Memorial Lecture: the immunology of the multiple sclerosis lesion. *Annals of neurology*, 36 Suppl, pp.S61–72.

Rao, S.M. et al., 1991. Cognitive dysfunction in multiple sclerosis. I. Frequency, patterns, and prediction. *Neurology*, 41(5), pp.685–691.

Reynolds, R. et al., 2011. The neuropathological basis of clinical progression in multiple sclerosis. *Acta neuropathologica*, 122(2), pp.155–170.

Rinaldi, F. et al., 2010. Cortical lesions and cognitive impairment in multiple sclerosis. *Neurological Sciences*, 31(S2), pp.235–237.

Rinaldi, F. et al., 2011. Natalizumab prevents the accumulation of cortical lesions in relapsing remitting multiple sclerosis: a preliminary report. *Neurological Sciences*, 31 Suppl 3(S3), pp.317–320.

Rinaldi, F. et al., 2012. Natalizumab strongly suppresses cortical pathology in relapsing-remitting multiple sclerosis. *Multiple sclerosis, 18(12), pp.1760-1767.*

Roosendaal, S. et al., 2009. Accumulation of cortical lesions in MS: relation with cognitive impairment. *Multiple Sclerosis*, 15(6), pp.708–714.

Ropele, S. et al., 2011. MRI assessment of iron deposition in multiple sclerosis. *Journal of magnetic resonance imaging : JMRI*, 34(1), pp.13–21.

Rossato, G. et al., 2010. Cerebral distribution of white matter lesions in migraine with aura patients. *Cephalalgia : an international journal of headache*, 30(7), pp.855–859.

Rovaris, M. et al., 2000. MRI correlates of cognitive dysfunction in multiple sclerosis patients. *Journal of neurovirology*, 6(S2), pp.S172-5.

Rudick, R.A. et al., 2009. Gray-Matter Injury in Multiple Sclerosis. *The New England journal of medicine*, 361(15), pp.1505–1506.

Runia, T.F. et al., 2012. Lower serum vitamin D levels are associated with a higher relapse risk in multiple sclerosis. *Neurology*, 79(3), pp.261–266.

Sailer, M., 2003. Focal thinning of the cerebral cortex in multiple sclerosis. *Brain : a journal of neurology*, 126(8), pp.1734–1744.

Saip, S. et al., 2007. Fabry disease mimicking multiple sclerosis. *Clinical neurology and neurosurgery*, 109(4), pp.361–363.

Samson, R.S. et al., 2013. Sulcal and gyral crown cortical grey matter involvement in multiple sclerosis: A magnetisation transfer ratio study. *Multiple Sclerosis and Related Disorders*, 2, pp.204-212.

Scalfari, A. et al., 2010. The natural history of multiple sclerosis: a geographically based study 10: relapses and long-term disability. *Brain : a journal of neurology*, 133(Pt 7), pp.1914–1929.

Schedel, J. et al., 2010. Cerebral lesions in patients with connective tissue diseases and systemic vasculitides: are there specific patterns? *Annals of the New York Academy of Sciences*, 1193, pp.167–175.

Schmierer, K et al., 2004. Magnetisation transfer ratio and myelin in postmortem multiple sclerosis. Annals of Neurology , 56(3), pp. 407 - 415.

Schmierer, K. et al., 2010. High field (9.4 Tesla) magnetic resonance imaging of cortical grey matter lesions in multiple sclerosis. *Brain : a journal of neurology*, 133(3), pp.858–867.

Schumacher, G.A. et al., 1965. Problems of experimental trials of therapy in Multiple Sclerosis: report by the panel on the evaluation of experimental trials of therapy in Multiple Sclerosis. *Annals of the New York Academy of*

Sciences, 122, pp.552–568.

Seewann, A. et al., 2009. Translating pathology in multiple sclerosis: the combination of postmortem imaging, histopathology and clinical findings. *Acta neurologica Scandinavica*, 119(6), pp.349–355.

Seewann, A. et al., 2011. Imaging the tip of the iceberg: visualization of cortical lesions in multiple sclerosis. *Multiple sclerosis (Houndmills, Basingstoke, England)*, 17(10), pp.1202–1210.

Seewann, A. et al., 2012. Postmortem verification of MS cortical lesion detection with 3D DIR. *Neurology*, 78(5), pp.302–308.

Serafini, B. et al., 2004. Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis. *Brain pathology (Zurich, Switzerland)*, 14(2), pp.164–174.

Sharma, R. et al., 2001. Grey matter abnormalities in multiple sclerosis: proton magnetic resonance spectroscopic imaging. *Multiple Sclerosis*, 7(4), pp.221–226.

Simon, B. et al., 2010. Improved in vivo detection of cortical lesions in multiple sclerosis using double inversion recovery MR imaging at 3 Tesla. *European Radiology*, 20(7), pp.1675–1683.

Simon, J.H., 2006. Brain atrophy in multiple sclerosis: what we know and would like to know. *Multiple Sclerosis*, 12(6), pp.679–687.

Simon, J.H, 2012. Very early MS -- insights from MRI. *Multiple sclerosis (Houndmills, Basingstoke, England)*, 18(10), pp.1372–1376.

Sinnecker, T. et al., 2012. Distinct lesion morphology at 7-T MRI differentiates neuromyelitis optica from multiple sclerosis. *Neurology*, 79(7), pp.708–714.

Smith, A., 1982. Symbol digit modalities test: Manual. Los Angeles: Western Psychological Services, *Manual.*

Snell, R., 1997 Chapter 1: Introduction and Organisation of the Nervous System. In *Clinical Neuroanatomy for Medical Students*. Lipincott Raven, pp. 76-89.

Snell, R., 1997a Chapter 15: Structure and Functional localisation of the Cerebral Cortex. In *Clinical Neuroanatomy for Medical Students*. Lipincott Raven, pp. 275–295.

Sobel, R.A. et al., 2008. Chapter 20: Demyelinating diseases In: G*Greenfields Neuropathology,* 8th edition, pp.1513 -1594.

Solomon, A.J. et al., 2013. Misdiagnosis of multiple sclerosis: frequency, causes, effects, and prevention. *Current Neurology and Neuroscience Reports*, 13(12), p.403.

Sperling, R.A. et al., 2001. Regional magnetic resonance imaging lesion burden and cognitive function in multiple sclerosis: a longitudinal study. *Archives of neurology*, 58(1), pp.115–121.

Stadelmann, C. et al., 2008. Cortical pathology in multiple sclerosis. *Current opinion in neurology*, 21(3), pp.229–234.

Stankiewicz, J. et al., 2007. Iron in chronic brain disorders: imaging and neurotherapeutic implications. *Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics*, 4(3), pp.371–386.

Steiner, G., 1952. Environmental studies in multiple sclerosis. *Neurology*, 2(3), pp.260–262.

Strober, L. et al., 2009. Sensitivity of conventional memory tests in multiple sclerosis: comparing the Rao Brief Repeatable Neuropsychological Battery and the Minimal Assessment of Cognitive Function in MS. *Multiple Sclerosis*, 15(9), pp.1077–1084.

Stys, P.K, 2004. Axonal degeneration in multiple sclerosis : is it time for neuroprotective strategies? *Ann Neuol* 55 pp 601-603

Summers, M. et al., 2008. Cognitive impairment in relapsing-remitting multiple sclerosis can be predicted by imaging performed several years earlier. *Multiple sclerosis (Houndmills, Basingstoke, England)*, 14(2), pp.197–204.

Sundström, P. et al., 2004. An altered immune response to Epstein-Barr virus in multiple sclerosis: A prospective study. *Neurology*, 62(12), pp.2277–2282.

Tallantyre, E.C. et al., 2009. A comparison of 3T and 7T in the detection of small parenchymal veins within MS lesions. *Investigative radiology*, 44(9), pp.491–494.

Tallantyre, E.C. et al., 2010. 3 Tesla and 7 Tesla MRI of multiple sclerosis cortical lesions. *Journal of magnetic resonance imaging : JMRI*, 32(4), pp.971–977.

Tedeschi, G. et al., 1999. Diffuse central neuronal involvement in Fabry disease: a proton MRS imaging study. *Neurology*, 52(8), pp.1663–1667.

Thompson, A.J. et al., 1990. Patterns of disease activity in multiple sclerosis: clinical and magnetic resonance imaging study. *BMJ : British Medical Journal*, 300(6725), p.631-634.

Tiemann, L. et al., 2009. Cognitive decline in multiple sclerosis: impact of topographic lesion distribution on differential cognitive deficit patterns. *Multiple Sclerosis*, 15(10), pp.1164-1174.

Tintoré, M. et al., 2003. New diagnostic criteria for multiple sclerosis: application in first demyelinating episode. *Neurology*, 60(1), pp.27–30.

Traboulsee, A.L. et al., 2006. The role of MRI in the diagnosis of multiple sclerosis. *Advances in neurology*, 98, pp.125–146.

Trapp, B.D. et al., 1998. Axonal Transection in the Lesions of Multiple Sclerosis. *The New England journal of medicine*, 338(5), pp.278–285.

Trapp, B.D. et al., 1999. Neurodegeneration in Multiple Sclerosis: Relationship to Neurological Disability. *The Neuroscientist*, 5(1), pp.48-57.

Trapp, B.D. et al., 2008. Multiple Sclerosis: An Immune or Neurodegenerative Disorder? *Annual Review of Neuroscience*, 31(1), pp.247–269.

Trapp, B.D. et al., 2009. Virtual hypoxia and chronic necrosis of demyelinated axons in multiple sclerosis. *Lancet neurology*, 8(3), pp.280–291.

Trip, S.A. et al., 2005. Imaging in multiple sclerosis.*Journal of Neurology, Neurosurgery and Neuropsychiatry,* 76(S3), pp.iii111-iii18.

Tsunoda, I. et al., 2002. Inside-Out versus Outside-In models for virus induced demyelination: axonal damage triggering demyelination. *Springer seminars in immunopathology*, 24(2), pp.105–125.

Tur, C. et al., 2011. Complementary roles of grey matter MTR and T2 lesions in predicting progression in early PPMS. *Journal of Neurology, Neurosurgery & Psychiatry*, 82(4), pp.423–428.

Turetschek, K. et al., 1998. Double inversion recovery imaging of the brain: Initial experience and comparison with fluid attenuated inversion recovery imaging. *Magnetic resonance imaging*, 16(2), pp.127–135.

Van der Elst, W. et al., 2006. The Stroop Color-Word Test: Influence of Age, Sex, and Education; and Normative Data for a Large Sample Across the Adult Age Range. *Assessment*, 13(1), pp.62–79.

van Horssen, J. et al., 2007. The Blood-Brain Barrier in Cortical Multiple Sclerosis Lesions. *Journal of neuropathology and experimental neurology*, 66(4), pp.321–328.

van Veluw, S.J. et al., 2012. In vivo detection of cerebral cortical microinfarcts with high-resolution 7T MRI. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*, 33(3), pp.322–329.

van Waesberghe, J.H. et al., 1998. Patterns of lesion development in multiple sclerosis: longitudinal observations with T1-weighted spin-echo and magnetisation transfer MR. *AJNR. American journal of neuroradiology*, 19(4), pp.675–683.

van Waesberghe, J.H. et al., 1999. Axonal loss in multiple sclerosis lesions: magnetic resonance imaging insights into substrates of disability. *Annals of neurology*, 46(5), pp.747–754.

van Walderveen, M.A. et al., 2001. Hypointense lesions on T1-weighted spin-echo magnetic resonance imaging: relation to clinical characteristics in subgroups of patients with multiple sclerosis. *Archives of neurology*, 58(1), pp.76–81.

Vercellino, M. et al., 2005. Grey matter pathology in multiple sclerosis. *Journal of neuropathology and experimental neurology*, 64(12), pp.1101–1107.

Vercellino, M. et al., 2009. Demyelination, Inflammation, and Neurodegeneration in Multiple Sclerosis Deep Gray Matter. *Journal of neuropathology and experimental neurology*, 68(5), pp.489–502.

Viglietta, V. et al., 2004. Loss of functional suppression by CD4+CD25+ regulatory T cells in patients with multiple sclerosis. *The Journal of experimental medicine*, 199(7), pp.971–979.

Vrenken, H. et al., 2006a. Whole-Brain T1 Mapping in Multiple Sclerosis: Global Changes of Normal-appearing Gray and White Matter. *Radiology*, 240(3), pp.811–820.

Vrenken, H. et al., 2006b. Altered diffusion tensor in multiple sclerosis normal-appearing brain tissue: cortical diffusion changes seem related to clinical deterioration. *Journal of magnetic resonance imaging : JMRI*, 23(5), pp.628–636.

Wattjes, M.P. et al., 2007. Double Inversion Recovery Brain Imaging at 3T: Diagnostic Value in the Detection of Multiple Sclerosis Lesions. *American Journal of Neuroradiology*, 28(1), pp.54–59.

Wattjes, M.P. et al., 2009. High field MRI in the diagnosis of multiple sclerosis: high field-high yield? *Neuroradiology*, 51(5), pp.279–292.

Wegner, C. et al., 2003. A new view of the cortex, new insights into multiple sclerosis. *Brain : a journal of neurology*, 126(Pt 8), pp.1719–1721.

Wegner, C. et al., 2006. Neocortical neuronal, synaptic, and glial loss in multiple sclerosis. *Neurology*, 67(6), pp.960–967.

Wegner, C. et al., 2009. Gray matter pathology and multiple sclerosis. *Current Neurology and Neuroscience Reports*, 9(5), pp.399–404.

Weinshenker, B.G. et al., 1989. The natural history of Multiple Sclerosis : A geographically based study *Brain* 112 (Pt 6), pp.1419–1428.

Westbrook, C., 2011. Chapter 1: Basic Principles. In *MRI in Practice,* 4th edition. pp. 8–10.

Yousry, T.A. et al., 1997. Comparison of MR pulse sequences in the detection of multiple sclerosis lesions. *AJNR. American journal of neuroradiology*, 18(5), pp.959–963.

Zang, Y.C.Q. et al., 2004. Increased CD8+ cytotoxic T cell responses to

myelin basic protein in multiple sclerosis. *Journal of immunology (Baltimore, Md. : 1950)*, 172(8), pp.5120–5127.

Zeis, T. et al., 2008. Normal-appearing white matter in multiple sclerosis is in a subtle balance between inflammation and neuroprotection. *Brain : a journal of neurology*, 131(1), pp.288–303.

Zhou, X.J., 2004. Basic Pulse Sequences. In M. A. Bernstein, K. F. King, & X. J. Zhou, eds. *Handbook of MRI Pulse Sequences*. Phase Sensitive Inversion Recovery. pp. 616–620.